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Some thirty authors have contributed to this book. Many of them are well known for their original contributions in the fields of biochemistry or food technology. They come from laboratories at academic centres, at government research institutions and at food factories. The book thus represents a piece of scientific collaboration by experts selected without any consideration other than their ability to assemble information and give it accurate expression. The authors have brought together all the knowledge at present available about the composition of the foods, and therefore the diets, of the British people. This has involved a detailed examination of seven main foods or groups of foods and the recording of innumerable data giving their chemical compositions.

The widest possible view has been taken as to the meaning of the words "chemical composition"; besides including vitamins, amino-acids and other constituents of less clear function (nucleo-tides, nitrogenous bases and so on), authors have given attention to the physical properties of the foods and also to the effect on them of cooking and processing. Based on a series of lectures given in war-time Britain, the seven parts of this book have much more than an ephemeral value.

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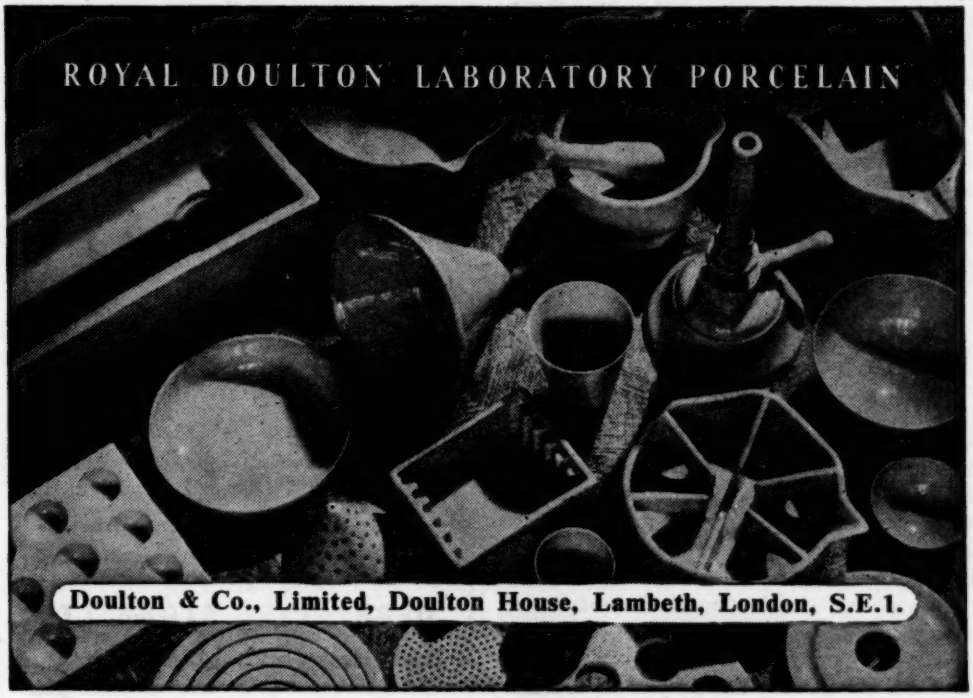

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and filing many forms. In all, he personally wrote or filed more than 7,000 letters and forms which were sent to draft boards, government officials, and others interested in and responsible for the operation of the Selective Service System. This prodigious effort, more than any other factor, contributed to his untimely death.

Whitmore had long been interested in petroleum. Before coming to Penn State he had studied the sulphur compounds in petroleum, and his work in aliphatic chemistry was applied in many ways to new petroleum products or refining methods. While at Penn State he and his colleagues synthesised approximately 300 different hydrocarbons: these pure materials were invaluable as reference standards in many entirely new petroleum developments. He was director of the work being done at Penn State for American Petroleum Institute Research Projects 42, 43B, and 46 which were concerned respectively with high-molecular-weight hydrocarbons, the origin of petroleum, and hydrocarbons for spectrometer calibrations. His work on the rearrangement of organic compounds was very important in the manufacture of special high octane super-fuels for military aircraft. From 1929 he and his colleagues in the Petroleum Refining Laboratory had been carrying out a comprehensive research and development programme, as a result of which more is now known about the composition of Pennsylvania petroleum than any other crude oil in the world. He was active in helping the Pennsylvania petroleum industry to obtain methods for characterising and identifying Pennsylvania lubricants in order to protect the public from unfair trade practices, and was a vigorous proponent of the application of scientific and technical developments to the efficient utilisation of Pennsylvania petroleum. He guided the Schools' research and development work towards the modernisation of the state's oil industry, the adaptation of new processes to the refining of Pennsylvania petroleum, and the use of chemicals and additives for improving the quality of the products.

Apart from the fraternity organisations, a number of learned and professional societies honoured Dean Whitmore. The American Association for the Advancement of Science elected him vice-president in 1932. He was a member of the American Institute of Chemical Engineers, the Chemical Society of London, Deutsche Chemische Gesellshaft, American Academy of Arts and Sciences, the American Institute, the National Institute of Social Sciences, the Franklin Institute, the Cosmos Club of Washington, D.C., and the Chemists Clubs of New York and Chicago. He was elected president of the Pennsylvania Chemical Society in 1940, and in 1928 was appointed a director of the Institute of Chemistry.

His spontaneity, enthusiasm, sense of humour, and boundless energy made him the best of companions. He was always cheerful, inspiring, and even-tempered. His friends will always remember him for his unfailing interest in and continual devotion to their welfare.

Whitmore was born in North Attleboro, Mass., on October 1st, 1887. He was the son of Frank Hale and Lena Avilla Thomas Whitmore. In 1914, he married Marion Gertrude Mason of Cambridge, Mass., who survives with four children.

M. R. Fenske.

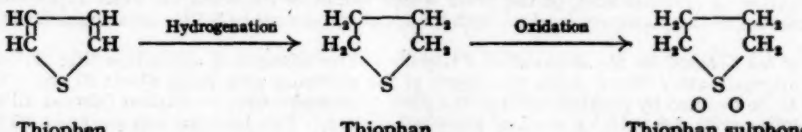
---

216. *Studies in the Detoxication of Catalyst Poisons. Part VI. Use of a Revivable Filter-column.*

By E. B. Maxted.

Since the detoxication of thiophen and of certain other types of catalyst poisons is greatly facilitated if the poison can be treated in an adsorbed state, a modification in procedure, involving the periodic detoxication of an adsorbent filter-column, has been developed. This avoids the necessity for a repeated detoxication treatment.

In Part V of the present series (*J.*, 1947, 624) an account has been given of the detoxication of thiophen in technical benzene and other systems by the two-stage process:



Thiophen (toxic). Thiophan (toxic). Thiophan sulphone (non-toxic).

in which the impure benzene is first subjected to a short (2—3 mins.) preliminary hydrogenation before the addition of an oxidising detoxicant, the hydrogenation step being necessary for the

production of a derivative more easily oxidisable than the original thiophen. It was found that the detoxication was in practice largely controlled by the degree of completeness which could be attained in the first stage; for, although thiophen in benzene tends to undergo preferential hydrogenation by virtue of its preferential adsorption on the catalyst, any very rapid hydrogenation is effectively limited to the amount of thiophen which can be initially adsorbed on the amount of catalyst used, since any subsequent interchange of thiophan for as yet unhydrogenated thiophen only takes place very slowly by reason of the toxic nature and consequently long adsorbed life of the product. The subsequent oxidation of the thiophan to its sulphone presents no difficulty; and this oxidation process, on account of the non-toxicity and short adsorbed life of the sulphone, leaves the surface of the catalyst free either for the adsorption of fresh thiophen or, if all the thiophen has undergone conversion, for the normal adsorption and hydrogenation of the benzene. Consequently, although the toxicity of very small concentrations of thiophen in benzene could be completely suppressed by a single treatment of the above nature, the detoxication of more heavily poisoned benzene, containing thiophen in excess of that which can be initially adsorbed on the small quantity of catalyst used, required a several times repeated application of this two-stage treatment before the whole of the thiophen ceased to be toxic. The following modification in procedure avoids the necessity for this repetition.

As an alternative to detoxication by the conversion of a poison into a non-toxic derivative which does not need to be removed, an impure system can obviously be freed from poisons by adsorption methods, for instance by being shaken with a suitable preliminary charge of catalyst, which is then removed; although, by reason of the partition even of strong poisons between the adsorbed and the free phase (*J.*, 1938, 2071), several successive treatments, each with a fresh charge of catalyst, would be necessary for the substantially complete removal of poison, unless a large excess of catalyst were used, even if, as with thiophen, the partition coefficient is strongly in favour of adsorption. If, however, a procedure is adopted in which the impure system is filtered through a stationary adsorbent of appreciable length, this undesirable operation of the partition effect against the complete removal of the poison in one treatment should be obviated, since the solution should become progressively weaker in poison as it passes through the adsorbent column. Further, if the column, after being used up to a stage at which it begins to allow poison to pass through, could be revivified periodically by treatment with a detoxicant, in such a way as to convert the adsorbed poison into a non-toxic form, it appeared that this use of a revivable filter might constitute a useful alternative method for freeing impure substances from poisons, since the column, when once set up, could by reason of its revivification be used indefinitely. The present short paper describes its application to benzene containing thiophen; but it should also be of interest for other poisons. Further, since the impure substance which is being prepared for hydrogenation does not, as in the general method used in previous papers, come into contact with an oxidising detoxicant—which is applied only to the spent column—this revivable filter-column method should be of particular use for the purification of substances, such as phenols, which cannot be exposed to an oxidation treatment.

Experimental.

Preparation of the Revivable Filter.—This preparation is given in detail, since it illustrates the method which has been adopted in this laboratory for the revival of spent catalysts. The platinum-black used for the adsorbing column did not consist of freshly prepared metal but of spent catalyst (from a platinum residue bottle) which had been previously used for miscellaneous poisoning work. About 10 g. of this were first washed with acetic acid, to remove most of the organic matter, then suspended in a further 100 c.c. of acetic acid and detoxicated by adding 30 c.c. of dilute aqueous sodium molybdate solution, containing 6×10^{-5} g.-atom of Mo per c.c., followed by 5 c.c. of 100-volume hydrogen peroxide added in 1-c.c. portions. Since all the catalyst had previously been used for hydrogenation reactions, no preliminary hydrogenation was necessary. The platinum was then washed with water and dried at 100°. A small sample (0·075 g.) was taken for testing and was found to have an activity, for the hydrogenation of crotonic acid, of the order which would be expected for fresh unpoisoned platinum. The remainder of the platinum (10·1 g., with an apparent volume of 8·1 c.c.) was used for the adsorption work.

Use of the Column for the Removal of Thiophen.—The adsorption apparatus (Fig. 1) consisted of a U-tube, approximately 70 cm. high, the length of the platinum plug being about 27 cm. The technical benzene to be purified by passage through the plug contained 0·09% of sulphur (almost all as thiophen), corresponding with $2·8 \times 10^{-5}$ g.-mol. of thiophen per c.c. This benzene was used as a 25% solution in acetic acid.

The degree to which the thiophen was removed as a result of the passage of the benzene through the platinum column was followed by taking successive samples from the outlet tap, the hydrogenation rate of these samples being compared with that given by a stock of sulphur-free benzene which had been

made, as a reference substance, by the dehydrogenation of sulphur-free *cyclo*hexane over palladium (Zelinsky, *Ber.*, 1911, **44**, 3121).

The standard system used in these hydrogenation tests consisted of 0·5 c.c. of benzene, dissolved in a total volume of 10 c.c. of acetic acid, and 0·1 g. of a stock platinum catalyst, the charge being hydrogenated under standardised conditions in a shaker at 50°. Under these conditions the sulphur-free benzene made from *cyclo*hexane absorbed hydrogen at a steady rate of 7·6 c.c. per minute, the corresponding rate with the untreated technical benzene being only 0·7 c.c. per minute. The hydrogenation rate of this benzene after passage through the adsorbing column was found also to be about 7·6 c.c. per minute, the observed rates for successive samples—expressed as a percentage of the rate given by the sulphur-free reference stock—being shown in Fig. 2. It will be seen that the

FIG. 1.

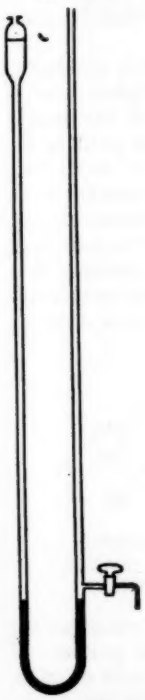
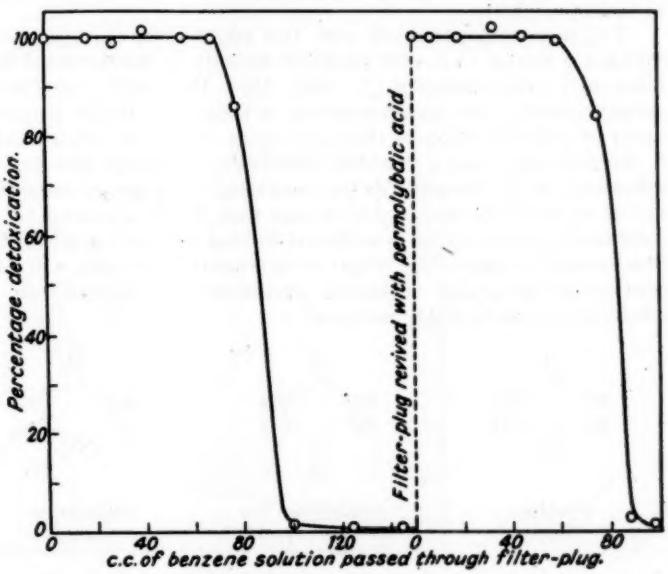


FIG. 2.



hydrogenation quality of the benzene was equal to that of the reference sample up to a stage corresponding with the passage of a total of about 70 c.c. of the 25% solution through the column, after which the rate fell rather steeply to that of the original benzene owing to the saturation of the column with thiophen. The flow of benzene was then discontinued; and the platinum plug, after being washed with a little acetic acid, was revived by hydrogenation followed by treatment with a permolybdate under the general conditions already described in connection with its preparation from spent platinum. On renewing the flow of impure benzene, successive samples were again found to be free from thiophen, as is shown by the second part of the curve; and the process of periodic revivification could apparently be repeated indefinitely, in that—since the present work—it has been found possible to employ the same column as a standard apparatus for the purification of many substances, including the preparation of the sulphur-free pyridine used in the following paper.

The author wishes to thank the Department of Scientific and Industrial Research for a grant.

UNIVERSITY OF BRISTOL. [*Received, August 19th*, 1947.]

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217. *Studies in the Detoxication of Catalyst Poisons. Part VII. The Self-poisoning Effect in the Hydrogenation of Pyridine.*

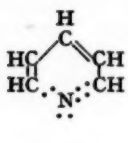
By E. B. MAXTED and A. G. WALKER.

The influence of the electronic state of the nitrogen atom in pyridine and in quaternary pyridinium compounds as a factor in inducing, respectively, a relatively low or a relatively high hydrogenation velocity has been considered from the standpoint of catalytic toxicity; and it has been shown that the low hydrogenation rate of free pyridine is probably a self-

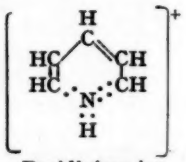
poisoning effect which tends to vanish if pyridinium ions are hydrogenated, owing to the shielded state of the nitrogen atom. This conception of nitrogen as a potentially toxic element seems to predict the catalytic toxicity of dry ammonia; and it has been found that very carefully dried ammonia dissolved in a water-free non-ionising solvent such as *cyclo*hexane acts as a poison towards a platinum catalyst in the liquid-phase hydrogenation of *cyclo*hexene.

PYRIDINE, even when free from sulphur, is known to undergo catalytic hydrogenation at a considerably lower rate than, for instance, benzene under similar conditions; and, since the nature of its unsaturated bonds does not differ greatly from those in aromatic hydrocarbons, it appears probable that its low hydrogenation rate may be an effect connected with the influence of its nitrogen atom. It has also been noticed by various workers that the successful hydrogenation of substances containing basic nitrogen has in some cases been facilitated by using an acidic catalyst.

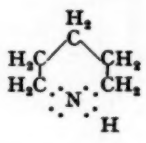
The present paper deals with this effect from the standpoint of the electronic conditions which are known to govern catalytic toxicity or non-toxicity in compounds of sulphur or other inherently toxic elements (*J.*, 1940, 252; 1941, 132; see also previous papers of the present series), namely, the correspondence of toxicity with the presence of free electron pairs in the outer or valency orbits of the toxic atom or, on the other hand, the disappearance of toxicity if the derivative has a shielded structure. Although nitrogen is not generally regarded as an inherently toxic element like the remaining members of its periodic group (phosphorus, arsenic, antimony and bismuth), it will be seen that, if the possession of a free electron pair by a suitably combined nitrogen atom is sufficient to lead to a strong adsorption by a metallic catalyst, then free pyridine or piperidine might be to some degree toxic, whereas the corresponding quaternary compounds generally, including pyridinium or piperidinium ions, should be less strongly adsorbed and accordingly non-toxic:



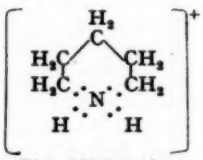
Pyridine (toxic).



Pyridinium ion (non-toxic, shielded structure).



Piperidine (toxic).



Piperidinium ion (non-toxic, shielded structure)

On these grounds, the slow rate of hydrogenation of free pyridine might be a self-poisoning effect due to the obstructively long adsorbed life—which is characteristic of all poisons—of piperidine on the catalytic surface: further, if the hydrogenation is carried out in acid solution, such that non-toxic pyridinium and piperidinium ions are present in place of the free bases, the self-poisoning effect should disappear and the hydrogenation of pyridine should proceed at the much higher rate which would be expected from the nature of its unsaturated bonds.

The above views have been confirmed experimentally. It has been shown that: (i) Free pyridine is an effective catalyst poison in the hydrogenation of unsaturated substances generally; *e.g.*, small quantities of sulphur-free pyridine added in the hydrogenation of *cyclo*hexene exert a toxic effect the magnitude of which is of the order of one-tenth of that exerted by the addition of an equivalent quantity of thiophen. Pyridine would accordingly be expected to act as a poison in its own hydrogenation. (ii) The rate of hydrogenation of sulphur-free pyridine can be raised to over eight times its normal value by the addition of a regulated quantity of an aqueous acid in such a way as to provide a pyridinium ion for the hydrogenation in place of free pyridine. Curves have been obtained showing the conditions required for a maximum hydrogenation velocity.

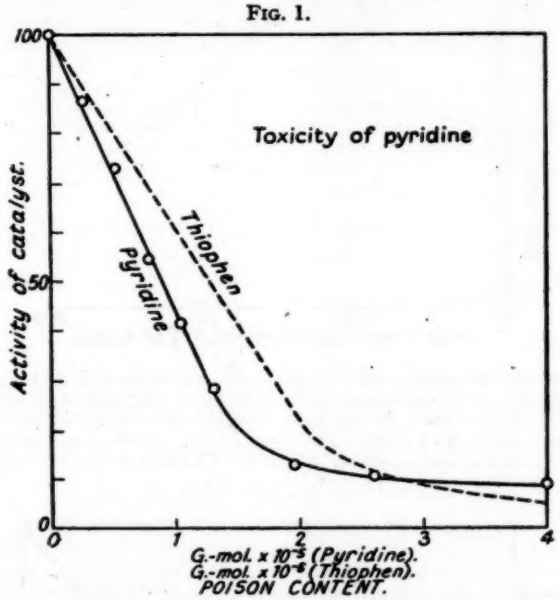
This conception of the toxic nature of nitrogen in free pyridine also raises the interesting question of the toxicity of free ammonia. Ammonium salts, as well as solutions of ammonia in water and the ammonium ion generally, are of course known to be non-toxic to metallic hydrogenation catalysts; but it has now been shown that carefully dried ammonia in a water-free non-ionising solvent such as *cyclo*hexane acts as a fairly strong catalyst poison in the hydrogenation of a non-acidic unsaturated substance such as *cyclo*hexene. The catalytic toxicity of dry free ammonia, contrasted with the known non-toxicity of the ammonium ion, agrees respectively with the non-shielded and the shielded electronic configuration of the nitrogen atoms and appears to confirm the inherently toxic nature of unshielded nitrogen in compounds of suitable structure.

Experimental.

Pyridine as a Catalyst Poison.—In order to establish the toxic nature of pyridine as an ordinary catalyst poison in a reaction involving the hydrogenation of a further unsaturated substance, the pyridine used must obviously be free from sulphur compounds and other extraneous poisons: accordingly, a stock of sulphur-free pyridine was prepared from the purest available pyridine (containing originally 0·015% of total sulphur) by the adsorption-column method described in Part VI (preceding paper). For the determination of toxicity *cyclo*hexene was taken as a convenient unsaturated substance, small known quantities of the pure pyridine, in *cyclo*hexane solution, being added to a standard hydrogenation system which consisted in each case of 5 c.c. of a 2N-solution of *cyclo*hexene in *cyclo*hexane, 0·05 g. of stock platinum catalyst, and a further 5 c.c. of *cyclo*hexane, including that added with the poison. The hydrogenation tests were carried out in a shaker at 27°.

It will be seen from the results plotted in Fig. 1, in which for purposes of comparison a corresponding toxicity curve for thiophen has also been included, that pyridine acts as a catalyst poison of moderate

Fig. 1.



strength, its toxicity being of the order of one-tenth of that of thiophen. This difference in the order of the toxicity of pyridine and of thiophen, when applied to the hydrogenation of the poisons themselves, explains why pyridine can be hydrogenated without great difficulty, even if the reaction is rather slow, whereas thiophen is hydrogenated far more slowly unless an unusually large proportion of catalyst is used (Mozingo *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 2092) so that a relatively large fraction of the thiophen taken is present in an adsorbed state (see also Part V). The difference in the order of the toxicity also explains why pyridine can be successfully freed from sulphur poisons by filtration through a column of platinum-black.

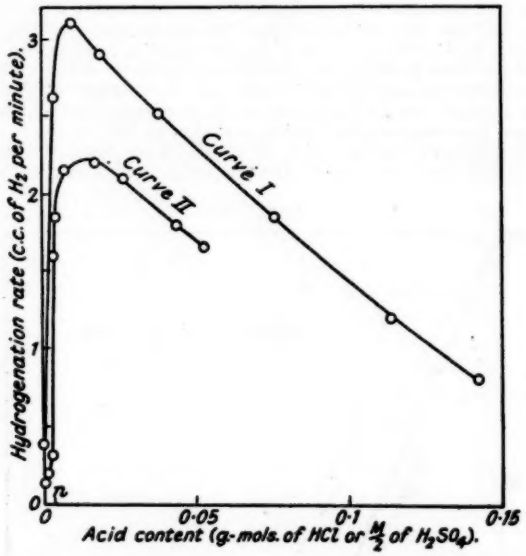
Hydrogenation of Pyridine.—The low hydrogenation rate of pyridine compared with that of benzene is shown in the following table, which refers to comparative tests with specimens of pyridine and benzene which had previously been freed from sulphur and other extraneous poisons with platinum-black. The hydrogenation rate of sulphur-free *cyclo*hexene is also included in order to show the still higher hydrogenation rate of an ethylenic bond, in a substance of similar structure, compared with that of the aromatic bonds in benzene. The tests were carried out in a shaker, at 27° and at atmospheric pressure, with similar charges consisting in each case of 10 c.c. of a N-solution of the unsaturated substance and 0·05 g. of stock platinum catalyst.

Unsaturated substance.	Solvent.	Hydrogenation rate, in c.c. of H_2 per min.
*cyclo*Hexene	*cyclo*Hexane	22·1
Benzene	,,	4·2
Pyridine	,,	0·33
Pyridine	Water	0·39

It will be noticed that, even in aqueous solution—in which there is some formation of pyridinium ions but still an appreciable concentration of free toxic pyridine—the hydrogenation rate of pyridine remains very low if no acid is present. It may also be noted that, in making up N-solutions of the various unsaturated substances for these comparative tests, the normality was, for equivalence in strength from a hydrogenation standpoint, based on the hydrogen value in such a way that the standard 10 c.c. hydrogenation charge required in each case the same volume (*ca.* 240 c.c.) of hydrogen for saturation. Accordingly, the N-*cyclo*hexene was molar, whereas the benzene and pyridine, both of which are capable

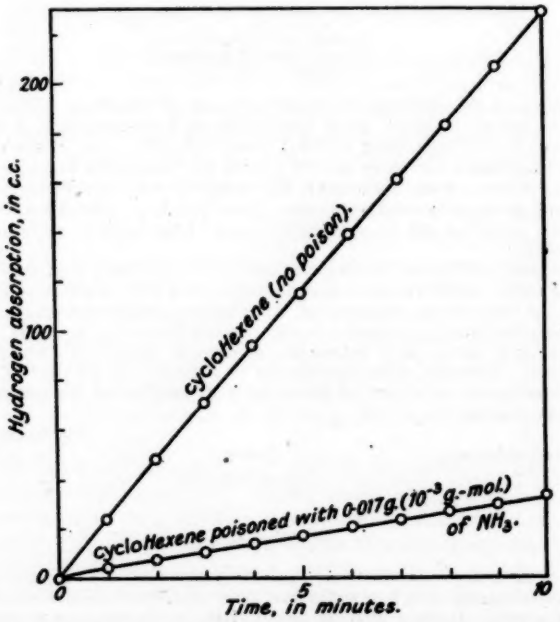
of absorbing three molecules of hydrogen per molecule of unsaturated substance, were one-third molar. The question of relative strengths is however not of great importance, since all these hydrogenation reactions are approximately of zero order.

FIG. 2.



The suppression of the self-poisoning effect in the hydrogenation of pyridine was studied by adding various amounts of sulphuric or hydrochloric acid to aqueous pyridine solutions, the composition of the 10-c.c. hydrogenation charges used for the tests being as already given save that the solvent was water containing a known amount of acid. The form of the curve obtained by plotting the hydrogenation rate against the acid content is shown in Fig. 2. Curve I of this figure relates to the addition

FIG. 3.



of sulphuric acid to sulphur-free pyridine. Curve II refers to a preliminary series of runs in which hydrochloric acid was added to a specimen of technical pyridine containing 0·024% of total sulphur. It will be seen that in each case the hydrogenation velocity rises very steeply as the acid present approaches the neutralisation point (marked *n* on the acid axis) but that the maximum velocity occurs

at an acid concentration which is considerably beyond that required for the neutralisation of the pyridine present. The general form of the curve shows, moreover, in addition to the expected rate-increasing effect of the addition of acid, the presence of an adverse factor, the weight of which increases continuously with an increase in the acid present. The nature of this adverse factor is not at present clear; and further work appears to be necessary before the subsequent fall in the rate can be explained. This should not have its origin in the presence of the increasing quantities of undissociated pyridinium or piperidinium sulphate or hydrochloride which would result from an increased acid content: moreover, the effect does not, as far as can be seen at present, seem to be due to the introduction of an extraneous poison with the acid; and the solutions are probably too dilute for the progressive cloaking of the catalyst by the deposition of an insoluble deposit. It is hoped to discuss this form of the curve further in a later paper.

Dry Ammonia as a Catalyst Poison.—It may be noted that gaseous ammonia, which has already been almost completely dried by passage through quicklime or solid sodium hydroxide, may be further dried with phosphoric oxide, since it is—unlike ammonia in the presence of water—a neutral gas when in a dry state. Accordingly, a solution of dry ammonia in *cyclo*hexane was made by passing into this solvent a current of pure ammonia, the final drying of which had been carried out with phosphoric oxide, the strength of this solution being adjusted so that 5 c.c. contained 0·017 g. (10^{-3} g.-mol.) of ammonia.

For the examination of the toxiclty of this dry ammonia towards a platinum catalyst in the hydrogenation of a neutral unsaturated substance, the hydrogenation rate, at 27°, of a charge consisting of 5 c.c. of a N-solution of *cyclo*hexene in *cyclo*hexane, 5 c.c. of the *cyclo*hexane solution of ammonia, and 0·05 g. of stock platinum-black was compared with the rate for a similar charge containing no ammonia. The results of these comparative tests are shown in Fig. 3, from which the catalytic toxicity of the ammonia will be seen.

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UNIVERSITY OF BRISTOL. [*Received, August 19th,* 1947.]

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218. *The Structures of Hantzsch's Isomeric Diazocyanides. A Reaffirmation of the Structural as opposed to the Geometrical Viewpoint, with a Critical Review of the Properties of the Alleged* cis-*Azobenzene.*

By HERBERT H. HODGSON.

The chemical evidence upon which Hodgson and Marsden recently proposed that Hantzsch's *syn*- and *anti*-diazocyanides are related as *iso*- and *n*-cyanides respectively is upheld as being of fundamental importance for the solution of the structural problem. It is emphasised that chemical analogies are against the geometrical hypothesis and in favour of a structural isomerism. Physical data require that the compound examined shall retain its structure during the examination, whereas the identical sets of data recently published by Le Fèvre and by Sutherland indicate interconversion from the unstable to the stable forms of the diazocyanides before the recorded measurements. The structure assigned to the alleged *cis*-azobenzene is not in harmony with many of the properties of this compound, and its analogy with the *syn*-diazocyanides is denied.

HODGSON and MARSDEN (*J.*, 1944, 395) have already submitted considerable chemical evidence in favour of the *iso*nitrile and the nitrile structure for Hantzsch's aryl *syn*- and *anti*-diazocyanides, respectively. The above paper followed one (*J.*, 1943, 470) in which Hantzsch's *syn*- and *anti*-diazosulphonates were claimed to possess sulphite and sulphonate structures respectively, and subsequently (*J.*, 1945, 207) chemical evidence was submitted for the structural isomerism of Hantzsch's *syn*- and *anti*-diazoates. Recently, however, Anderson, Le Fèvre, and Savage (*J.*, 1947, 445) have expressed their view that Hodgson and Marsden's chemical evidence for the *syn*- and *anti*-diazocyanides is equivocal and not incompatible with the hypothesis of geometrical isomerism, while they assert that recorded dipole moments and refractivity data are also against Hodgson's conclusions. A second paper by Anderson, Bedwell, and Le Fèvre (*J.*, 1947, 457) dealing with magnetic optical rotatory powers and diamagnetic susceptibilities is also submitted in support of their geometrical viewpoint. Further, an investigation of the vibration spectra of 4-bromo- and 2 : 4 : 6-tribromo-benzenediazocyanides by Sheppard and Sutherland (*J.*, 1947, 453) is claimed to prove conclusively that these isomers cannot be related as *iso*cyanide and cyanide but are both cyanides. These claims are now examined.

The Original Objective of the Oxime Analogy.—It is perhaps of interest to recall that Hantzsch's work in this field had the primary and initially the only purpose of establishing the structures of the diazoates, which were distinguished by the ability of one of them to couple immediately with phenols and amines and of the other to resist coupling. For the purpose, Hantzsch made

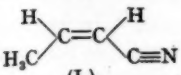
use of the geometrical analogy with the oximes, whose *syn*- and *anti*-forms (later proved by Meisenheimer and Lange, *Ber.*, 1924, **57**, 282, to have the opposite configurations assigned to them by Hantzsch) seemed to furnish an appropriate parallel. Hantzsch thus reared the whole of his theory upon the fundamental proposition that the difference between the oximes and the diazo-compounds was merely the substitution of nitrogen for carbon, and that the series of so-called normal and *iso*-diazo-compounds must be derived from two isomeric diazohydroxides.

Energy States of the Alleged Geometrical Isomers.—According to Saunders (" The Aromatic Diazo-Compounds ", Arnold, 1936, p. 185), the *anti*-form must be at a lower energy level than the *syn*-form, and this has been confirmed by Le Fèvre and Vine (*J.*, 1938, 431), who report the heat evolved in the transformation of *p*-bromobenzene *syn*- to *anti*-diazocyanide as 21—22 kcals./mole, *i.e.*, of the same order as the difference in bond energies between the N–N and the C–N bond. The rapidity of transformation of *syn*- to *anti*-compounds by heat has been stressed by previous investigators (cf. Hantzsch and Schultz, *Ber.*, 1895, **28**, 666; Le Fèvre and Vine, *loc. cit.*; Hodgson and Marsden, *loc. cit.*). Moreover, Hodgson and Marsden doubted whether Hantzsch had ever been in possession of a pure *syn*-sulphonate or *syn*-cyanide. This rapidity of transformation readily explains the physical data assembled by Hantzsch, as well as the identity of the absorption spectra as found by Dobbie and Tinkler (*J.*, 1905, **87**, 276) for the *syn*- and *anti*-sulphonates and for the *syn*- and *anti*-cyanides. In contrast, the absorption spectrum of Hantzsch's diazonium cyanide was quite different (cf. Hantzsch and Lifschitz, *Ber.*, 1912, **45**, 3011). Notwithstanding these facts, Saunders (*op. cit.*, p. 195) comments that the only unchallenged item (of this great controversy) is the spectroscopic evidence, and that this must tell heavily on the credit side of Hantzsch's account, whereas in fact the evidence could not be more definite against him.

Discussion of Hantzsch's Data.—When Hantzsch was challenged as to the diazonium character of the *syn*-sulphonates, he was quick to reply with the visual colour argument, *viz.*, that benzenediazonium salts are colourless, whereas the *syn*- and *anti*-compounds are highly coloured. It is incredible, therefore, that such outstanding differences in visual colour, *viz.*, that the *syn*- and *anti*-sulphonates are red- and yellow-orange respectively, while in contrast the *syn*- and *anti*-cyanides are yellow and red respectively, should not have been seriously considered by Hantzsch, especially when he obtained identity of absorption spectra and then proceeded to argue therefrom for the geometrical isomerism of these compounds. It is of great interest, therefore, that Hantzsch and Reddelien record (" Die Diazo-Verbindungen ", 1921, p. 50) that the *syn*-diazosulphonates have a tendency in aqueous solution to afford ions of the diazonium sulphite, and, although not stated, it would appear that Hantzsch's view of the mechanism of transformation of *syn*- to *anti*-sulphonate is *via* the diazonium sulphite; since the formation of the *syn*-sulphonate must be from diazonium and sulphite ions initially, it is surely reasonable to conclude from Hantzsch's experiment that the diazo-sulphite and not the sulphonate is formed originally, and is, in fact, the alleged *syn*-sulphonate. When the original papers of Hantzsch were examined, no record whatever could be found to indicate that, at the conclusion of the physical experiments, the conditions of the compounds investigated were ever tested to ascertain whether any transformations had taken place or not. It would have been a simple matter for Hantzsch *et al.* to have found whether the final compound coupled or not, and thereby to have established quantitatively the extent, if any, of the change from *syn*- to *anti*-sulphonate or cyanide. It is irrational to argue diversity of structure from identity of test, and the writer reaffirms his former standpoint that the data are for the *anti*-compounds alone.

The Paper by Sheppard and Sutherland (loc. cit.).—The outstanding feature of this investigation is the almost complete identity of the " finger-prints " for the pairs of *syn*- and *anti*-isomers examined. No better experimental evidence could have been given that the substances were identical, *i.e.*, that transformation had taken place from *syn*- to *anti*-form in each case. The specimens examined were prepared elsewhere, and no time is stated for the interval between preparation and measurement. Further, no temperatures are given, no experiments are recorded to show that the compounds had remained unchanged either before or after the experiments, and no preliminary investigation of the effect of infra-red radiation on these remarkable compounds was made. It is the Hantzsch situation over again. Moreover, in the discussion from analogy, alkyl and aryl cyanides are compared with their isomeric *iso*cyanides, so that in the former case a C–C bond connects the CN group with the rest of the molecule, and, in the latter case, a C–N bond is concerned; in none of the diazocyanides is a C–C bond in question. The analogies, therefore, are not as complete as these authors have asserted, and for the $R{-}N{=}N{-}\overset{+}{N}\overset{-}{C}$ structure, *i.e., for an* N–N *bond, no analogies are given.* This matter of

an irrelevant C–C bond runs throughout Sutherland's discussion, *e.g.*, for *cis*- and *trans*-crotononitrile (I) and (II), and for the conjugation of the C≡N bond with the N=N bond he has to rely on his own prediction. The fact that the CN frequency is in exactly the same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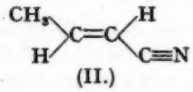
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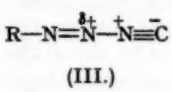
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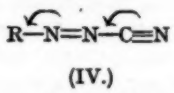
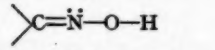
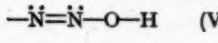

(IV.)

position in all the diazocyanides is, in the author's opinion, very strong evidence that *anti*-forms are present throughout. With regard to the prediction for diazo*iso*cyanides, the fact appears to have been ignored that this is a case of Pauling's " like adjacent charges " (III) (" The Nature of the Chemical Bond ", 1940, pp. 109, 210), in which resonance is feeble, as shown by the yellow colour of the compounds as well as by their instability (ease of coupling), whereas in the nitrile compounds where resonance is facile (IV), the colours are deep red, and coupling prevented. Sutherland's statement, therefore, that the spectroscopic evidence against the *iso*cyanide theory can be regarded as overwhelming, is at least very premature; neither has Sutherland proved conclusively, as he asserts, that Hantzsch's diazocyanides are *both* cyanides.

The Papers by Anderson, Le Fèvre, and Savage, and by Anderson, Bedwell, and Le Fèvre (loc. cit.).—In the preamble to the first paper, justice is not done to the arguments of Hodgson and Marsden (*loc. cit.*), inasmuch as Le Fèvre's dipole-moment data are not given the prominence suggested, while the foundational experimental evidence based on the behaviour of N–C and N–N bonds *is completely ignored* by Le Fèvre. The example of a diazo-carbonamide, R·N:N·CO·NH_2, was chosen by Hodgson and Marsden as typical, since this compound resists fission of its N–C bond by coupling agents. When the carbonamide is converted into an *iso*cyanate, R·N:N·NCO, by the Hofmann or the Lossen reaction, coupling immediately becomes possible, since the N–C bond has now been changed to an N–N bond. Instead, Le Fèvre *et al.* focus their criticism on the Grignard experiments, which after all constitute contributory data only, since their failure would not invalidate the main argument in the slightest degree. Le Fèvre *et al.* also state that such properties as relative speeds of coupling, ionisation, etc., do not necessitate the possession by *syn*-compounds of the readily broken N–N bond and by *anti*-compounds of the more stable C–N link, while similar, although less marked, contrasts are often shown by geometrical isomers. The author emphatically asserts that the difference between the immediate coupling of the *syn*-compounds and its absence with *anti*-compounds is not to be described as a relative speed of coupling, and that no differences between geometrical isomers, a long list of which is cited by Le Fèvre, can even compare with this remarkable difference of immediate scission at the link between the diazo- and the cyanogen groups in the case of the *syn*-compounds. Moreover, the author refuses to accept an analogy between a saturated carbon bonded to an unsaturated nitrogen, as in the oximes (V), and two

(V.) 


(VI.)

unsaturated nitrogens of the diazo-group in the diazo-compounds (VI), and he asserts that there is no similarity in the sense of the present discussion between oximes and diazo-compounds. Finally, the alleged existence of azobenzene in *cis*- and *trans*-forms is used as evidence by Le Fèvre *et al.* for the possibility of geometrical isomerism about the –N=N– system, whereas when *cis*-azobenzene was claimed to have been discovered the analogy with the *syn*-diazocyanides was invoked in its favour. In view of the fact that many of the properties exhibited by the alleged *cis*-azobenzene are anomalous, the author had always refused to believe in its existence until more unequivocal evidence was forthcoming. These anomalies will be discussed later, but meanwhile, the author objects to *cis*-azobenzene being invoked to establish the possibility of existence of *syn*-diazocyanides and *vice versa*.

In their second set of comments on dipole moments, Le Fèvre *et al.* repeat the analogies of Sutherland where the cyanides have all C–C bonds and the *iso*cyanides all C–N links; nowhere is an N–N link quoted. Instead, it is stressed that the moment of phenyl cyanide is only slightly greater than that of phenyl *iso*cyanide, an argument irrelevant to the whole issue. It is true that in the dipole-moment data differences appear between the *syn*- and the *anti*-forms, but Le Fèvre's arguments are based on the unique and constant structures of the *syn*-compounds, whereas the methods of preparation cited in the papers by Le Fèvre *et al.* indicate mixtures to have been obtained; otherwise, what is meant by the statement " By the following modification . . . *greatly improved* specimens of the unstable forms are obtained ", and again, " materials with melting points as high as ever previously recorded "? The author is convinced

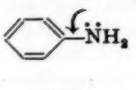
from the renewal of his experimental work in this field that, not only had Hantzsch always worked with impure specimens of *syn*-diazosulphonates and cyanides, but that Le Fèvre *et al.* have given no proof whatever that in the cases of their *syn*-diazocyanides were they really ever in possession of unique compounds.

It might be argued that differences obtained for other physical properties such as dipole moment, etc., and ascribed by the author to mixtures of *cis*- and *trans*-, should also affect the absorption spectra, but this would depend on the relative amounts of each isomeride present, and on the sensitiveness of the test. It must be realised that the various quantitative experiments of Le Fèvre and Sutherland were not carried out simultaneously or even standardised, and that no one has yet stated the actual amount of the *syn*-compound present in any of its preparations; apparently Le Fèvre unwarrantably assumes his starting material to be 100% pure (cf. Le Fèvre and Vine, *loc. cit.*, who deduce the rates of isomerisation of *cis*- to *trans*-diazocyanides from dipole-moment data in benzene at 25°, *i.e.*, of compounds prepared at or below — 10°, which are very unstable at 0°, and isomerise spontaneously at *ca.* 50°). In actual fact, therefore, the starting compound must have been a mixture, and this would account for the rate of isomerisation being sufficiently slow to enable its measurement at 25°.

The Grignard Reactions.—In all Hodgson and Marsden's experiments the ether used was first tested by hydrazone formation with 4-nitro- and 2 : 4-dinitro-phenylhydrazine for the presence of any acetone or aldehyde as impurity, since the former is a possibility *ab initio* and the latter by slow oxidation during long storage. It is of course possible that a *syn*-cyanide can oxidise ethyl ether to acetaldehyde just as diazonium salts oxidise ethyl alcohol to this aldehyde, especially as the *syn*-diazocyanides behave in many respects like diazonium salts (cf. Stephenson and Waters, *J.*, 1939, 1796), and a diazonium structure would probably have been assigned to them long ago but for their pronounced colour and sparing solubility in water. The oxidation of alcohol by diazonium salts, however, is usually carried out under boiling conditions and over a time period much greater than that involved in these Grignard reactions, whereas the oxidation, if any, of ether by the *syn*-diazocyanides is not only more difficult than that of alcohol, but in addition would have to take place at 0° and during a few minutes. Hodgson and Marsden (*loc. cit.*) attributed their low yields (maximum 20%) of acetaldehyde to the rapid change of *syn*- to *anti*-diazocyanide, probably accelerated by the Grignard reagent itself, since in most cases no yields at all were obtained; and this circumstance is suggested for the failure of Le Fèvre in the benzaldehyde experiments. In the latter case, where phenylmagnesium bromide is used in place of methylmagnesium iodide, Le Fèvre should have obtained a measurable yield of acetaldehyde just as in the other experiments, since it is attributed by him to the oxidation of the ether by the *syn*-cyanide, whereas he reports practically no yield at all. The author maintains that Le Fèvre *et al.* have in nowise proved that the acetaldehyde reported by Hodgson and Marsden, and confirmed by them, did not come mainly from the interaction of the Grignard reagent with the *syn*-cyanide and subsequent decomposition of the complex formed. Finally, the same objection as was made earlier against the experiments of Hantzsch and of Sutherland must be made against all the physical measurements recorded for the unstable *syn*-cyanides—some of them at 20° and 25°—for substances which have to be prepared below — 10°. In fact, the most unstable of Hantzsch's *syn*-cyanides are dealt with as though their stability was in no doubt whatever, while calculations are based on the same irrelevant analogies for cyanides and *iso*cyanides possessing C–C and C–N bonds respectively.

An Argument for the Structural Viewpoint based on Analogy.—The strength of the C–N bond and its resistance to fission is an outstanding feature of all compounds in which it occurs, and, where resonance is possible, as in (VII), the bond is even strengthened. Only under conditions involving high temperatures and pressures can this bond be broken, *e.g.*, in alkyl and aryl *iso*cyanides and nitro-, nitroso-, and amino-compounds. Further, when a halogen is *ortho*- or *para*- to a nitro-group in a benzene or naphthalene derivative, it is the halogen which is displaced by anionoid reagents, *i.e.*, the C–Cl and not the C–N bond is ruptured. On the other hand, in such compounds as $R{\cdot}N{:}N{\cdot}CH_3$, $R{\cdot}N{:}N{\cdot}C_6H_5$, $R{\cdot}N{:}N{\cdot}CO{\cdot}NH_2$, and $R{\cdot}N{:}N{\cdot}CN$ (*anti*), no coupling occurs with alkaline β-naphthol; it is only in diazonium salts (VIII) that the bond is readily broken, and in these cases the simultaneous evolution of the nitrogen molecule is also necessary. The author does not accept the free-radical hypothesis of Hey and Waters for the decomposition of diazo-compounds in aqueous solution, which he considers to be unnecessary, and his reasons for this standpoint have already been given (Hodgson, this vol., p. 348). In fact, the only examples of a ruptured C–N bond without attendant complex decompositions are in the alleged *syn*-diazocyanides. On the other hand, all the diazo-compounds with N–O bonds couple immediately with alkaline β-naphthol, *e.g.*, $C_6H_5{\cdot}N{:}N{\cdot}OH$, $C_6H_5{\cdot}N{:}N{\cdot}O{\cdot}CH_3$,

$C_6H_5\cdot N{:}N\cdot O\cdot CO\cdot CH_3$ [and the diazo-oxides if they are assumed to have the formula (IX)]; similarly all the chain diazo-compounds with N–N bonds couple immediately under appropriate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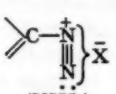
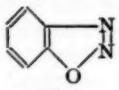

(VIII.)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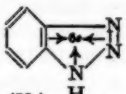
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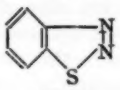
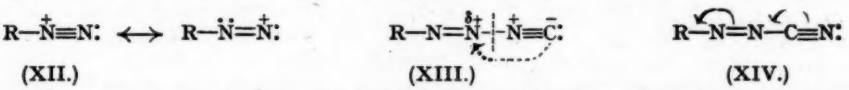

(XI.)

conditions, *e.g.*, $C_6H_5\cdot N{:}N\cdot NH\cdot C_6H_5$, $C_6H_5\cdot N{:}N\cdot N{:}CO$, and diazoimino-compounds, but the ring compounds such as (X) do not, though here we have an example of benzene-like stability produced by the existence of an aromatic sextet [cf. Robinson, "Outline of an Electrochemical (Electronic) Theory of the Course of Organic Reactions", Institute of Chemistry, 1932, p. 10]. Further, *all* the diazo-compounds with N–S bonds will not couple, *e.g.*, $C_6H_5\cdot N{:}N\cdot SO_3Na$ (*anti*), $C_6H_5\cdot N{:}N\cdot S\cdot CH_3$, $C_6H_5\cdot N{:}N\cdot S\cdot C_6H_5$, $C_6H_5\cdot N{:}N\cdot SO_2R$ and (XI), with the *single* exception of the alleged *syn*-sulphonates, *e.g.*, $C_6H_5\cdot N{:}N\cdot SO_3Na,K$ (*syn*). The *syn*-sulphonates and *syn*-cyanides thus stand out as the *sole* exceptions of their respective classes, so that *evidence for their existence should be overwhelming*, especially in view of the great anomaly. The alleged *syn*-sulphonates, however, have been shown beyond doubt to be sulphites (Hodgson and Marsden, *loc. cit.*), so that now the *syn*-diazocyanides stand alone.

The Validity of the Infra-red Spectra.—The evidence for the existence of *syn*-diazocyanides depends upon an *absolute* proof of the presence in them of the $>N{-}C{\equiv}N{:}$ group. It is obvious that two compounds which differ so much that their visual colours are yellow and red, respectively, must be in very different states of internal strain (cf. Saunders, *op. cit.*), and therefore should exhibit other outstanding differences in properties, including spectra whether visual or otherwise. Germane to this standpoint is a paper by Sutherland and Thompson (*Trans. Faraday Soc.*, 1945, **41**, 175), wherein it is stated (in italics) that "the infra-red spectrum of a chemical compound is probably the most characteristic property of that compound"; and (again in italics): "In principle, however, no two molecules other than a pair of optical isomers can have an exactly similar array of frequencies, and although two closely related molecules may have several absorption bands at identical wave-lengths, we may expect to find some spectral region in which differences can be found. In this sense the infra-red spectrum is a fingerprint of the molecule, and with structural isomers of relatively simple molecules, as well as with highly complex organic structures, this spectrum may well be the most characteristic physical property of the compound." Again (*ibid.*, p. 207), Le Fèvre asks "whether two molecules which are structurally identical but spatially different might have infra-red spectra *at least as unlike* as are the visible and ultra-violet spectra for such compounds", and in reply Thompson (*ibid.*, p. 208) states: "although information about the spectra of *cis*- and *trans*-isomers is accumulating, I do not think that we are yet in a position to predict on theoretical grounds how the spectra should differ, except perhaps in some simple cases. The mathematical treatment of complex molecules is not at present very satisfactory, mainly because little is known about the force fields in such molecules."

After such emphatic pronouncements in January, 1945, Le Fèvre *et al.* and Sheppard and Sutherland then present in July 1946 what really amount to identical fingerprints for sets of alleged *syn*- and *anti*-isomers of which the *syn*- have to be prepared at − 10°, and, in some compounds at least, commence to change into the *anti*-isomers forthwith. Even the identity of the fingerprint of the probably more stable tribromobenzene-*syn*-diazocyanide with that of its isomer cannot be claimed as supporting evidence for the very unstable *p*-nitro- and *p*-chlorobenzene-*syn*-diazocyanides; on the contrary the identities of the fingerprints for compounds which are known to isomerise spontaneously at 0° (Le Fèvre, *loc. cit.*) and below 0° (Hantzsch, *loc. cit.*), invalidate the conclusions which their investigators have drawn. It is reasonable, therefore, not only on grounds of analogy, but also because it accounts for all the chemical properties of the alleged aryldiazo-*syn*-cyanides, that their general formula should be, $R{-}N{=}\overset{+}{N}{-}\overset{-}{N}{\equiv}C$. It is also germane to the issue that in the cases of all the compounds which on Hantzsch's theory should be capable of geometrical isomerism, *e.g.*, $R\cdot N{:}N\cdot O\cdot CH_3$ (especially in view of the stress laid by Hantzsch, Saunders, Le Fèvre, and others on oxime analogy), $R\cdot N{:}N\cdot S\cdot CH_3$, $R\cdot N{:}N\cdot SO_2R$, and $R\cdot N{:}N\cdot NHR$, not a single one can be obtained in two forms, and that the only cases claimed are the *syn*-sulphonates (now proved to be non-existent) and the *syn*-cyanides. *It is therefore incredible that*, in view of the enormous relative stability of the *anti*-sulphonates and *anti*-cyanides, *a mere angular difference should produce such remarkable changes in chemical properties and in visual spectra.*

The Mode of Formation of the Aryldiazoisonitriles.—This may be envisaged as follows: on addition of potassium cyanide to the solution of the diazonium salt the cyanide anion will approach the diazonium kation (XII) at the nitrogen in accord with the approach of diazo-compounds to aniline, since the nitrogen atom has less affinity for a negative charge than carbon; the diazo*iso*nitrile (XIII) will thereby be formed initially, but will immediately commence to undergo a redistribution of electrical charge so that the N–N link becomes exceedingly weak from the presence of like positive charges on its members, and forthwith



(XII.) (XIII.) (XIV.)

commences to isomerise into the more stable diazocyanide (XIV), whose stability is greatly enhanced by the facile resonance that will now occur. During the *iso*nitrile stage, it is obvious that immediate coupling can occur with suitable reagents, but that in the cyanide condition coupling is now prevented. In the formation of the diazosulphites (*syn*-sulphonates) it is clear that the approach of the sulphite anion must be at an oxygen atom, and to give a sulphite initially.

There now remains the analogy with *cis*-azobenzene quoted by Le Fèvre *et al.* to show that geometrical isomerism about the $^{-}N{=}N^{-}$ system is possible.

A Critical Discussion of Some of the Properties of the Alleged cis-*Azobenzene.*—Since the geometrical isomerism of compounds containing the azo-group is restricted to that of *cis*- and *trans*-azobenzene (hereinafter referred to as *cis* and *trans*) the evidence for its existence ***must be unequivocal.*** The following discussion will show, however, that a double compound of azoxybenzene and hydrazobenzene exhibits far more convincingly many of the reactions given by *cis*. The data singled out for discussion are as follows:

(1) Ease of conversion of *cis* to *trans* is paralleled by ease of reaction between hydrazobenzene and azoxybenzene to give *trans*. Evaporation in a vacuum of an ice-cold chloroform solution of *cis* is sufficient to bring about some conversion into *trans*. Photochemical formation of *cis* from *trans* in the presence of water (Hartley, *J.*, 1938, 633) lends support to the view of an oxidation–reduction reaction for production of azoxy- and of hydrazo-benzene. Irradiation of absolutely dry ligroin solutions of *trans* in absence of air afforded no *cis*, whereas when water was present a deepening of colour resulted, indicating the formation of *cis*; this colour was discharged by desiccating agents such as anhydrous sodium sulphate with re-formation of *trans*. Contrary to these results, Hartley and Le Fèvre (*J.*, 1939, 531) have concluded that solutions in benzene of *cis* and of *trans* separately when illuminated by sunlight each give an equilibrium mixture containing 22% of *cis*; no compounds were isolated from these *trans* experiments, and whether the experiments were conducted in absence of air is not stated.

(2) Since *cis* has a dipole moment, its molecular weight of 179 in benzene (associating solvent) could be expected to be higher than that of 190 in acetic acid (dissociating solvent), whereas the opposite is the case (Hartley, *loc. cit.*), although the differences from 182 for azobenzene are small enough to be within experimental error from the standpoint of conversion to *trans* during the determinations.

(3) The red colour given by *cis* with concentrated sulphuric acid or hydrochloric acid is similar and peculiar to azoxybenzene, which has been ascribed by Wallach and Bellis (*Ber.*, 1880, **13**, 528) to isomerisation of azoxy- to hydroxyazo-benzene. Since *trans* gives only a yellow solution in concentrated sulphuric acid, the origin of the red colour given by *cis* is more than difficult to explain in the absence of auxochromes.

(4) The greater solubility of *cis* than of *trans* in polar solvents is more reasonably accounted for by the above double compound. Hartley (*loc. cit.*) records a much higher solubility for *cis* than for *trans* in 0·5N- and in N-hydrochloric acid, although he argues, notwithstanding, that the *cis* is not much, if at all, more basic than the *trans*. On Swientoslawski's hypothesis (*Bull. Soc. chim.*, 1924, **35**, 137; *Ber.*, 1929, **62**, 2034; *Annalen*, 1931, **491**, 273) that deformation of the azo-group should make it more acidic, *i.e.*, less prone to dissolve in acids (and this would also follow on electronic grounds), the greater solubility of *cis* in acids—or even Hartley's argument that the *cis* is not much, if at all, more basic than the *trans*—is evidence contrary to the hypothesis of a *cis*-structure.

(5) It is noteworthy that Cook (*J.*, 1938, 878) isolated what might be an azoxytoluene by long irradiation of *mm'*-azotoluene *in air*.

(6) Robertson (*J.*, 1939, 232) found that ordinary *trans*-azobenzene crystallised in the monoclinic system, that its *X*-ray spectrum was easily explained, and that two almost planar

centrosymmetrical molecules contributed to the asymmetrical unit. A quite different crystal structure was encountered with Hartley's *cis*-azobenzene, in that it was orthorhombic while the molecule had a two-fold axis but no centre of symmetry. To accommodate these results into a *cis*-azo-structure involved considerable ingenuity (cf. also Hampson and Robertson, *J.*, 1941, 409). Pauling (*op. cit.*, p. 221) writes in comment on the above data : " that steric interaction of the two rings in *cis* is so great that a coplanar configuration cannot be achieved since contact of the ortho-hydrogen atoms of the two rings prevents the molecule from assuming the coplanar configuration, and each phenyl group is rotated *ca.* 50° out of the coplanar orientation. Since coplanarity is an essential attribute of double-bond character, it is assumed that the amount of conjugation energy in *cis* is probably not greater than 2 or 3 kcals./g.-mol., and the difference in energy as determined by Hartley (*loc. cit.*) between *cis* and *trans* of 12 kcals./g.-mol. is taken as the conjugation energy for the *trans*-configuration ". This considerable departure from coplanarity with consequent strain in the molecule should also be accompanied by an appreciable difference in its absorption spectrum from that of the *trans* isomer, but such is not the case, for while the *cis* absorbs more light at the blue end of the spectrum, there is no displacement of the visible band although the ultra-violet absorption band is shifted towards a higher frequency (Le Fèvre and Vine, *loc. cit.*, p. 438). This relatively small difference in light absorption, which, so far as visible light is concerned, is even contrary to what should be expected from a highly strained molecule (*i.e.*, less instead of more blue), is in striking contrast with the absorption spectra differences exhibited by other geometrical isomers. For example, in the case of *cis*- and *trans*-di-*p*-tolylethylene, where the *trans* is completely changed by sunlight to the *cis* in *ca.* 2 hours (Martin, Bett, Romans, and Tidridge, *Trans. Roy. Soc. Canada*, 1940, **34**, 35), the *trans* is yellow with strong absorption at 4047 A., whereas the *cis* is colourless and quite transparent at 4047—3660 A., absorbing at 2536 A. only slightly. On analogy, therefore, the almost complete absence of resonance in *cis*-azobenzene as compared with the facile resonance in *trans*-azobenzene should have been accompanied by very dissimilar absorption, and if the analogy with the *syn*-diazocyanides as adopted by Hartley and by Le Fèvre is pressed (cf. *J.*, 1947, 446), the visual colour should at least have been yellow whereas *cis*- is visually redder than the *trans*-azobenzene, and likewise the deepening of colour in the irradiation experiments, instead of lightening, is the qualitative test of the formation of *cis*-azobenzene. The analogies quoted by Le Fèvre and Vine (*loc. cit.*) are invalid, since the geometrically isomeric diazoates and diazosulphonates do not exist. Is it possible that *cis*- and *trans*-azobenzene are the only geometrical isomers in which the *more* strained member is *more* bathochromic than the practically unstrained isomer, since the contrary should be the case if the structure assigned to *cis*-azobenzene is correct? In the double-compound alternative above, the reddening of the yellow colour of the azoxybenzene component would be accounted for by relief of the strain imposed by its dipole through association with the anionoid hydrazobenzene.

(7) Finally, it is of great interest to find that the surprisingly large value of 12 kcals./g.-mol. found, and so commented upon, by Hartley for the conversion of solid *cis*- at 21° to solid *trans*- at 21° is of the same order as that for the heat of reaction between azoxy- and hydrazo-benzene when computed from their published (Beilstein) heats of combustion at constant pressure, namely, 10·7 kcals per g.-mol. of *trans*-azobenzene formed. Further, with respect to analytical data, the following are the calculated percentage values for the elements in azobenzene and (in parentheses) for the double compound : C, 79·1 (75·4); H, 5·5 (5·75); N, 15·4 (14·7); from which it is obvious that only the value for carbon is discriminating, and conversion even in part would bring this value within the limits of experimental error.

It follows, therefore, that weighty evidence exists against the structure assigned to the alleged *cis*-azobenzene, and since it and the *syn*-diazocyanides are the only examples left of geometrical isomerism about the $-\dot{N}=\dot{N}-$ system, further data of an unequivocal character must be forthcoming to establish the validity of the geometrical hypothesis in these cases. Meanwhile, the author adheres to his original view that the *syn*-diazocyanides are *iso*nitriles, and that the structure of *cis*-azobenzene has not been unequivocally established.

Conclusions.—(1) The data of Le Fèvre *et al.* (*loc. cit.*) and of Sheppard and Sutherland (*loc. cit.*) are challenged on the ground that no tests appear to have been made to prove that interconversion of *syn*- to *anti*- during the course of the experiments had *not* taken place. The identity of the spectroscopic data for both isomers is a convincing proof of identity of compound, particularly in view of their facile interconversion; in any event it is no evidence of the similarity of geometrically isomeric forms.

(2) In a compound so highly strained as the alleged *syn*-diazocyanide, wherein rupture of the C–N link occurs with the greatest ease as in coupling with phenols and amines, compared with

the *anti*-isomer which resists coupling, other marked differences should also exist, notably in their spectra; *e.g.*, the visual colours being different, why should the visual spectra be alike if interconversion had not taken place?

(3) Irrelevant analogies are given by the above authors for the interpretation of their data, and the real analogies that C–N and N–S bonds universally resist rupture, whereas N–O and N–N bonds split readily, are now emphasised.

(4) In view of the ephemeral character of the compounds discussed, it would appear that chemical is of far more importance than physical evidence for the problem in hand.

(5) The properties of the alleged *cis*-azobenzene are in many respects so anomalous that doubt must be expressed as to its *cis*-azo-structure.

(6) To formulate the isomeric diazocyanides as *both* cyanides with a C–N link, is to accept a structure for one of them (the yellow one) whose chemical behaviour contradicts it. The author therefore adheres to the *iso*nitrile structure, $R-N{=}N-\overset{+}{N}{\equiv}\overset{-}{C}$, for these very reactive compounds.

The experimental work alluded to in the section dealing with *cis*-azobenzene was carried out during 1938—1940 in collaboration with Drs. W. Davey and E. Marsden. Adverse climatic conditions during the succeeding summers have prevented further work despite frequent attempts. The author, however, feels that the present occasion requires a statement of his views, since the analogy of *cis*-azobenzene with the *syn*-diazocyanides has recently been stressed.

TECHNICAL COLLEGE, HUDDERSFIELD. [*Received, August 14th*, 1947.]

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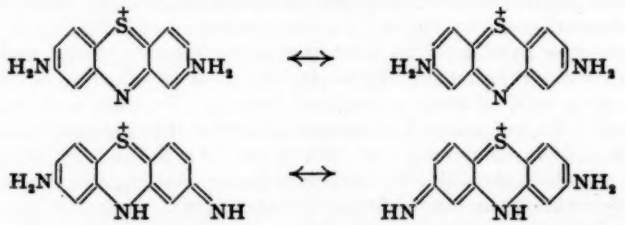
219. *The Preparation of 2 : 2′-Dichloro-4 : 4′-dinitrodiphenyl Sulphide and 2′-Chloro-4 : 4′-dinitro-2-aminodiphenyl Sulphide, with Some Derivatives and Attempts at Cyclisation to Thiazines.*

By HERBERT H. HODGSON, DOUGLAS P. DODGSON, and ERNEST W. SMITH.

The above preparations had for their objective the ultimate formation of 2 : 8-diaminothiodiphenylamine (2 : 8-diaminophenothiazine) in which, on oxidation, the thiazine ring would play the reversed rôle to that in Lauth's violet and methylene-blue. Attempted cyclisations by an imino-bridge, however, failed, and this is tentatively attributed to lack of co-planarity of the benzene rings in the various sulphides used.

THE work now reported had for its objective the preparation of thiazine compounds by cyclisation of *oo′*-disubstituted diphenyl sulphides, where, in the dyes therefrom, the sulphur, now in the *o*- and *p*-positions to the nitrogen, would play the predominant chromophoric rôle, *i.e.*, it would be a constituent of all the quinonoid structures possible, *e.g.*:



When the starting compound is a substituted diphenylamine derivative, cyclisation by thionation readily takes place, as indicated by the direct thionation of diphenylamine itself when heated with sulphur alone at 250—300° (Bernthsen, *Annalen*, 1885, **230**, 77) or with sulphur and aluminium chloride at 135—160° (Ackermann, D.R.-P. 222879; Friedländer, **10**, p. 144), or in the various syntheses of methylene-blue. Unexpected difficulties, however, occurred when a diphenyl sulphide was the initial material, and so far the cyclisation objective has not been achieved. The following modes of approach have been tried: In the attempted preparation of 2 : 2′ : 4 : 4′-tetraminodiphenyl sulphide from 2 : 2′ : 4 : 4′-tetranitrodiphenyl sulphide by various reducing agents with possible cyclisation *en route*, the great ease of elimination of sulphur, especially in iron and tin reductions, became very evident, with *m*-phenylenediamine as the reaction-product instead of the expected 2 : 8-diaminothiodiphenylamine (cf. Hodgson and Dodgson, in the press). The reduction with zinc and acetic acid gave blue sulphur-containing complexes, whereas stannous chloride in acetic acid appeared to avoid both complex formation

and elimination of sulphur. Attempts to produce the imino-bridge by heating the newly prepared 2 : 2′-*dichloro*-4 : 4′-*dinitrodiphenyl sulphide*, with ammonia under pressure at 200°, or for 10 hours with potassium phthalimide at 200°, left the initial material unchanged in both cases. Unsuccessful cyclisation attempts from 4 : 4′-dinitro-2 : 2′-diaminodiphenyl sulphide are also recorded in the experimental section.

Ammonium sulphide appears to convert 2 : 2′ : 4 : 4′-tetranitrodiphenyl sulphide into the disulphide, possibly by air oxidation of the thiol produced by a process of hydrosulpholysis, $R_2S + H_2S \longrightarrow 2R{\cdot}SH$, analogous to hydrolysis, which seems to have occurred. Attempts at the cyclisation of this compound may therefore involve far more deep-seated changes than were expected.

It is of interest that Matsumura (*J. Amer. Chem. Soc.*, 1930, **52**, 3199) found that 2 : 2′ : 4 : 4′-tetra-aminodiphenyl ether could not be cyclised by heating the hydrochloride either alone or in the presence of zinc chloride in an atmosphere of carbon dioxide for 20 hours at 200°, while in the preparation of the tetra-amine, by reduction of the tetranitro-compound with stannous chloride, an appreciable amount of oxygen was liberated corresponding to the analogous elimination of sulphur from the sulphide analogue (*loc. cit.*). In striking contrast, there is the ready cyclisation of 2 : 2′-diaminodiphenyl to carbazole by heating it with an excess of 25% sulphuric or 15% hydrochloric acid in a sealed tube for 15 hours at 200° (Tauber, *Ber.*, 1891, **24**, 197), and the conversion of 2 : 2′-diaminodiphenylamine into phenazine by such simple oxidising agents as ferric chloride, hydrogen peroxide, or potassium permanganate (Eckert and Steiner, *Monatsh.*, 1914, **35**, 1153). These results would appear to indicate non-coplanarity of the benzene rings in the sulphide and ether above. Support for this view comes from the non-substantivity to cotton of the bisazo-dyes from 4 : 4′-diaminodiphenyl sulphide as compared with the affinity for cotton of those from 4 : 4′-diaminodiphenylamine (cf. Hodgson and Marsden, *J. Soc. Dyers and Col.*, 1944, **60**, 210, for coplanarity as a factor for substantivity). Further, while resonance of the whole molecule will account for coplanarity in a diphenylamine, it is prevented in an *oo*′-diaminodiphenyl sulphide, so that cyclisation with sulphur will be facilitated in the former and elimination of ammonia will be frustrated in the latter type of compound.

Finally, the preparation is now reported of 2-*chloro*-4 : 4′-*dinitro*-2′-*aminodiphenyl sulphide*, and an attempt was made to cyclise the acetyl derivative, with the object of effecting a diphenylamine-type of synthesis (Goldberg, *Ber.*, 1907, **40**, 4541), by heating it in nitrobenzene solution with potassium carbonate and cuprous chloride at 180° for 7 hours. None of the initial compound was left, but the brown unidentifiable substance gave an olive-green colour with concentrated sulphuric acid, thereby indicating some salt-forming character.

Experimental.

2 : 2′-*Dichloro*-4 : 4′-*dinitrodiphenyl Sulphide.*—*o*-Dichlorobenzene (11·3 c.c., b. p. 174·5°), prepared by the Sandmeyer reaction from *o*-chloroaniline, was dissolved in sulphuric acid (25 c.c., *d* 1·84), and powdered potassium nitrate (11 g.) then stirred gradually into the solution at 0°. When the addition was complete, the mixture was stirred for an hour without external cooling and poured on ice. The oily layer of 2 : 3- and 3 : 4-dichloronitrobenzene was separated and cooled to − 10°, after which the oily paste was drained at the pump to leave a colourless crystalline 3 : 4-dichloronitrobenzene. Alternatively, *o*-dichlorobenzene (7 c.c.) was stirred during 4 hours into a mixture of nitric acid (7 c.c., *d* 1·5) and sulphuric acid (9 c.c., *d* 1·84) at 0°; almost solid 3 : 4-dichloronitrobenzene then separated, and was worked up as above. The 3 : 4-dichloronitrobenzene crystallised from methanol in silky colourless needles, m. p. 43° (Beilstein and Kurbatov, *Annalen*, 1875, **176**, 41, give m. p. 43°). For the preparation of the sulphide, the solution of 3 : 4-dichloronitrobenzene (9·6 g.) in ethanol (70 c.c.) was mixed with one of sodium sulphide nonahydrate (6 g.) in ethanol (10 c.c.) and water (10 c.c.); the mixture, which immediately turned red, was refluxed for 2 hours, filtered hot, and the solid 2 : 2′-*dichloro*-4 : 4′-*dinitrodiphenyl sulphide* (6 g., 70% yield) washed with ethanol (50 c.c.) and water (100 c.c.), after which it crystallised from 80% acetic acid (charcoal) in long yellow needles, m. p. 166° (Found : Cl, 20·3. $C_{12}H_6O_4N_2Cl_2S$ requires Cl, 20·6%). Alternatively, 3 : 4-dichloronitrobenzene (2 g.), dissolved in ethanol (15 c.c.), was added to a hot solution of sodium sulphide nonahydrate (1·5 g.) in ethanol (7 c.c.), the mixture refluxed for 10 hours, and the 2 : 2′-dichloro-4 : 4′-dinitrodiphenyl sulphide filtered off and recrystallised as above; the filtrate from this experiment, on dilution with water, afforded 2-chloro-4-nitrophenetole (0·2 g.), 3 : 4-dichloroaniline (0·2 g., determined as 3 : 4-dichlorobenzeneazo-β-naphthol), and unchanged 3 : 4-dichloronitrobenzene (0·2 g.). When heated in a sealed tube with ethyl-alcoholic ammonia at 200° for 12 hours no amination occurred.

2 : 2′-*Dichloro*-4 : 4′-*dinitrodiphenyl sulphoxide* was obtained when the above sulphide was stirred at room temperature with nitric acid (*d* 1·5) for 30 minutes; it crystallised from glacial acetic acid on addition of water in pale buff needles, m. p. 184° (Found : S, 9·1. $C_{12}H_6O_5N_2Cl_2S$ requires S, 8·9%).

2 : 2′-*Dichloro*-4 : 4′-*dinitrodiphenyl sulphone* was formed when a solution of the sulphide above in the minimum of acetic anhydride was heated on the water-bath for 12 hours with a large excess of 30% hydrogen peroxide; it separated from 80% acetic acid in stellate clusters of colourless needles, m. p. 169° (Found : S, 8·6. $C_{12}H_6O_6N_2Cl_2S$ requires S, 8·5%).

2′-*Chloro*-4 : 4′-*dinitro*-2-*aminodiphenyl sulphide* was produced when a solution of 2-chloro-5-nitroaniline

(1·725 g.) in ethanol (30 c.c.) was treated with one of sodium sulphide nonahydrate (3·6 g.) and sodium hydrogen carbonate (1·26 g.) in water (20 c.c.), and the mixture heated on the water-bath under the reflux for 1 hour; a further addition of sodium hydrogen carbonate (1·26 g.) dissolved in ethanol (10 c.c.) and water (10 c.c.) was made, and the refluxing continued for 5 minutes, after which a solution of 3 : 4-dichloronitrobenzene (1·92 g.) in ethanol (20 c.c.) was added and the refluxing continued for 2 hours. After cooling, the solid was filtered off, washed with ethanol (50 c.c.) and water, and dried; it crystallised from glacial acetic acid (charcoal) in short yellow needles, m. p. 211° (Found: N, 13·1. $C_{13}H_9O_4N_2ClS$ requires N, 12·9%), which were quite insoluble in dilute or concentrated hydrochloric acid. On acetylation *2-chloro-4 : 4-dinitro-2-acetamidodiphenyl sulphide* was obtained; this crystallised from glacial acetic acid in pale yellow prisms, m. p. 187° (Found: N, 11·6. $C_{14}H_{10}O_5N_3ClS$ requires N, 11·4%).

Attempts to Cyclise 4 : 4′-Dinitro-2 : 2′-diaminodiphenyl Sulphide.—The following treatments were found to be ineffective. (*a*) Boiling with excess of concentrated hydrochloric acid for 3 hours. (*b*) Heating with excess of aqueous sulphuric acid (33% by vol.) on a water-bath for 3 hours, or with excess of aqueous acid (25% by vol.) at the boiling point for 2 hours. In the next experiments the initial material was charred completely. (*c*) Heating with excess of zinc chloride in an oil-bath at 200° for 30 minutes. (*d*) Heating with excess of sulphuric acid (67% by vol.) for 3 hours in an oil-bath at 200°. With conditions intermediate between these extremes, only indications of products that gave colour reactions were obtained. (*e*) Heating with aqueous sulphuric acid (50% by vol.) for 2 hours at the boiling point charred most the material, but dilution of the filtrate did not precipitate any initial substance. The sulphuric acid solutions, however, had a red colour, which was discharged by reduction with zinc but restored by subsequent addition of ferric chloride.

Action of Ammonia and Hydrogen Sulphide on 2 : 2′ : 4 : 4′-Tetranitrodiphenyl Sulphide.—The monosulphide (1 g.) was suspended in ethanol (35 c.c.), saturated with ammonia gas, and the mixture treated with hydrogen sulphide until dissolution occurred. The deep red solution was heated on the water-bath for 15 minutes, filtered, acidified, and the pale yellow precipitate extracted with glacial acetic acid; the residue was 2 : 2′ : 4 : 4′-tetranitrodiphenyl disulphide, which crystallised from pyridine or aniline in light yellow micro-crystals that exploded at *ca.* 300° (Claas, *Ber.*, 1912, **45**, 753, gives explosion point, 303°).

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220. *Studies on Phosphorylation. Part IV. Further Studies on the Use of Dibenzyl Chlorophosphonate and the Examination of Certain Alternative Phosphorylation Methods.*

By F. R. ATHERTON, H. T. HOWARD, and A. R. TODD.

Investigations directed to the improvement of phosphorylation procedures using dibenzyl chlorophosphonate as phosphorylating agent have been carried out. An improved method for the purification of dibenzyl phosphite and an alternative method for its conversion into dibenzyl chlorophosphonate are described. The action of tertiary bases on dibenzyl chlorophosphonate is discussed, and 2 : 6-lutidine is shown to have advantages over pyridine in the phosphorylation of alcohols with this reagent. *Dibenzhydryl phosphite* has been prepared, and from it, by reaction with carbon tetrachloride and amines, several substituted aminophosphonates have been obtained. Hydrogenolysis of benzyl esters of phosphoric acid, for which general conditions are given, proceeds, as expected, more readily than that of benzhydryl esters. Substituted diazomethanes can be used with advantage for the preparation of phosphites and phosphates, but the method is limited by the accessibility of the diazo-compounds.

IN Part I of this series (Atherton, Openshaw, and Todd, *J.*, 1945, 382) the preparation of dibenzyl chlorophosphonate and its application to the phosphorylation of amines and alcohols was described; the same reagent, prepared by a different route, has also been employed as a phosphorylating agent by Deutsch and Fernö (*Nature*, 1945, **156**, 604). The choice of this reagent was dictated largely by our intention to apply it in a variety of synthetic studies on nucleotides and other physiologically active phosphates and polyphosphates. In these studies it was necessary to have a reagent which would phosphorylate under mild conditions at low temperature and which would yield initial products from which the protecting groups could be removed readily by either hydrolysis or hydrogenolysis, since syntheses were envisaged in which these two methods would have to be applied at different stages. Dibenzyl chlorophosphonate possesses, from this point of view, advantages over most other reagents which have been employed for phosphorylation, the more so as it has been shown that partial debenzylation of the intital reaction products can be effected by either hydrogenolysis (Part I, *loc. cit.*) or hydrolysis (Baddiley and Todd, *J.*, 1947, 648). The technique of partial debenzylation offers a route to polyphos-

phates which has been utilised in the synthesis of adenosine-5′ pyrophosphate (adenosine diphosphate) (Baddiley and Todd, *loc. cit.*). Phosphorylation with dibenzyl chlorophosphonate has been widely employed in these laboratories, and in consequence considerable attention has been paid to the improvement of the method and its reduction to a routine procedure for general use. As described in Part I (*loc. cit.*), the procedure, although fairly satisfactory, had certain defects; first, purification of the dibenzyl phosphite used as starting material was rather tedious; secondly, the yields obtained in the phosphorylation of alcohols were only moderate; and thirdly, removal of benzyl groups from the initial products by hydrogenation was, at times, unaccountably slow. These points, among others, have been the subject of closer study.

In order to ascertain whether the complete decomposition of dibenzyl phosphite, which usually occurred on attempting to purify it by normal vacuum distillation, was due to the presence in the crude material of impurities produced in the reaction between phosphorus trichloride and benzyl alcohol, an alternative method of preparation was examined. Pallazo and Maggiacomo (*Gazzetta*, 1908, **38**, II, 115) showed that diethyl phosphite can be prepared by the action of diazoethane on phosphorous acid, the latter presumably reacting as the tautomeric phosphonic acid, $HPO(OH)_2$. Phenyldiazomethane reacted smoothly with phosphorous acid, but the product decomposed as before on attempted distillation by ordinary methods at 10^{-3} mm.; low temperature purification by the method already described (Part I, *loc. cit.*) gave, however, a high yield of pure dibenzyl phosphite. Since then the difficulty of distillation could not be attributed simply to by-products formed in the phosphorus trichloride route, the next possibility to be considered was that it might have its origin in the formation of traces of acidic material in the preliminary heating of the dibenzyl phosphite. Accordingly, crude dibenzyl phosphite was saturated with gaseous ammonia and filtered from the small amount of solid material formed; a small amount of *N*-methylmorpholine was then added and the mixture rapidly distilled under reduced pressure in a nitrogen atmosphere. This procedure, which is described in the experimental section, was found quite satisfactory, and by it large quantities of dibenzyl phosphite have been purified without difficulty. A possible reason for the formation of acidic material on heating dibenzyl phosphite, which seems to have been the source of our earlier difficulties, is mentioned later in this paper in connection with work on *dibenzhydryl phosphite*.

The preparation of dibenzyl chlorophosphonate by the action of chlorine on dibenzyl phosphite (Part I, *loc. cit.*) is satisfactory, although it has to be carried out below 0°. With the object of making available an alternative route which might proceed at room temperature, the use of sulphuryl chloride, a mild chlorinating agent, was examined. In preliminary experiments it was found that diethyl and di*iso*propyl phosphites were readily converted into the corresponding chlorophosphonates by being warmed with this reagent. The same conditions were unsuitable for dibenzyl phosphite; in this case a diluent had to be employed, and reaction then occurred at room temperature, giving dibenzyl chlorophosphonate in good yield. An observation made in the course of experiments aimed at the preparation of dialkyl chlorophosphonites is worthy of mention. Treatment of diethyl or di*iso*propyl phosphite with thionyl chloride yields not the chlorophosphonite but the corresponding chlorophosphonate, together with unidentified halogen-free products; the mechanism of this reaction is obscure and warrants further study.

Phosphorylation of amines by dibenzyl chlorophosphonate is almost quantitative, but phosphorylation of alcohols in presence of pyridine under the conditions described in Part I (*loc. cit.*) gives variable yields often as low as 50%. Dibenzyl chlorophosphonate can be heated to 55° for at least 1½ hours without noticeable decomposition, and it is quite stable to ethanol in the absence of tertiary bases. It seemed, therefore, that the comparatively low yields obtained on phosphorylating alcohols must be due to some reaction between the tertiary base, or its hydrochloride, and dibenzyl chlorophosphonate, or the initial product. The speed at which the reaction was carried out seemed to be one factor affecting the results, since dibenzyl chlorophosphonate gave a higher yield with *n*-butanol in presence of pyridine when the reaction was carried out within 3 hours at 40° than after 18 hours at room temperature. When *N*-methylmorpholine was substituted for pyridine in the reaction with *n*-butanol, the low temperature reaction gave a large amount of *N*-benzyl-*N*-methylmorpholinium chloride, and reaction at the higher temperature apparently a mixture of this substance with *N*-methylmorpholine hydrochloride; in neither case could the other products of reaction be hydrogenated. These observations suggest that the main reaction competing with that leading to dibenzyl *n*-butyl phosphate and *N*-methylmorpholine hydrochloride is one between base and reagent,

leading to *N*-benzyl-*N*-methylmorpholinium chloride and, presumably, benzyl metaphosphate according to the scheme:

$$R_3N + (Ph{\cdot}CH_2{\cdot}O)_2POCl \longrightarrow R_3\overset{+}{N}(CH_2Ph){\cdot}\overset{-}{O}{\cdot}PO(O{\cdot}CH_2Ph)Cl \longrightarrow R_3\overset{+}{N}(CH_2Ph)\overset{-}{Cl} + Ph{\cdot}CH_2{\cdot}O{\cdot}PO_2$$

Support for this view was provided by the observation that a high yield of *N*-benzyl-*N*-methylmorpholinium chloride is obtained when an equimolar mixture of dibenzyl chlorophosphonate and *N*-methylmorpholine is kept at room temperature. An analogous reaction almost certainly occurs, although doubtless to a lesser extent, in phosphorylations using pyridine, since it was found that dibenzyl chlorophosphonate is completely destroyed by pyridine in 2½ hours at 34°. It was evident that as a step towards increasing yields obtained in phosphorylating alcohols it would be desirable to find some tertiary base causing less destruction of dibenzyl chlorophosphonate than pyridine. Accordingly, several common tertiary bases were mixed with dibenzyl chlorophosphonate at room temperature, and the amount of reagent remaining after 1 hour was determined by treating the mixture with ammonia and weighing the dibenzyl aminophosphonate produced. The percentage yields of dibenzyl aminophosphonate were: pyridine, 58%; α-picoline, 70%; quinoline, 77%; dimethylaniline, 83%; 2 : 6-lutidine, 85%; quinaldine, 86%. A control experiment in absence of tertiary base gave a 90% yield of dibenzyl aminophosphonate. Of the three bases causing least decomposition in 1 hour, only 2 : 6-lutidine satisfied the requirements of availability and volatility for general use, and we have employed it with advantage in place of pyridine in many phosphorylations. With it, the yields of, *e.g.*, barium ethyl phosphate and barium glucose-6 phosphate were increased by some 15% over our earlier figures. The nature of the alcohol has, of course, an effect and in some cases—*e.g.*, *n*-butanol, 2′ : 3′-*iso*propylideneadenosine—the yields with 2 : 6-lutidine are not so markedly improved; nevertheless, 2 : 6-lutidine is, in general, preferable to pyridine as a tertiary base in alcohol phosphorylations. Even in the best cases examined by us, however, the yields obtained with alcohols still fall considerably short of those obtained in phosphorylating amines. The tendency for tertiary base hydrochloride produced in the reaction to debenzylate the initial reaction product is doubtless one adverse factor which operates. Another will obviously be the speed at which the alcohol employed undergoes reaction, since the stability of the quaternary phosphorylating intermediate will be low in presence of excess of tertiary base It is probable that, in order to get maximum yields, optimum conditions would have to be determined for each individual case, but the general procedure indicated in this paper may be taken as reasonably satisfactory.

In view of the difficulty occasionally encountered in our early work of removing benzyl groups from the dibenzyl phosphates obtained as initial products in phosphorylation, a careful study has been made of the various factors influencing hydrogenolysis. Hydrogenolysis proceeds best in ethanol or dioxan solution, preferably in presence of a little water. It is accelerated by traces of mineral acid and retarded by bases, and platinum, palladium, or nickel catalysts can be employed. For general use we recommend a palladium oxide catalyst in amount 2—5% of the material to be hydrogenolysed in presence of a trace of sulphuric acid. Under these conditions no difficulty has been encountered, and hydrogen uptake proceeds at a rate of 30—100 c.c./min. A point of interest is that, where 2 : 6-lutidine is used as tertiary base in phosphorylating alcohols, the reaction products are quite colourless and hydrogenation proceeds smoothly and rapidly even in the absence of any acid.

Since it would sometimes be useful to prepare substituted phosphates containing groups other than benzyl, which could nevertheless be removed by selective hydrogenolysis, dibenzhydryl phosphite was prepared by the action of diphenyldiazomethane on phosphorous acid. Dibenzhydryl phosphite, unlike dibenzyl phosphite, is a high-melting solid. It is, however, very unstable, and on being kept or on recrystallisation it gradually decomposes with formation of dibenzhydryl ether; decomposition is very rapid on heating. Since one would expect, on general grounds, that dibenzyl phosphite, although more stable, would resemble the dibenzhydryl ester in properties, it is probable that the former will undergo at higher temperatures a similar decomposition yielding dibenzyl ether. Such a decomposition would involve the simultaneous formation of acidic material and may explain the tendency of dibenzyl phosphite to decompose violently on distillation as mentioned above. The chlorination of dibenzhydryl phosphite was examined, but no dibenzhydryl chlorophosphonate could be obtained; it was probably formed initially but was so unstable that it decomposed immediately. By treatment of the ester with carbon tetrachloride and the appropriate amine (Atherton, Openshaw, and Todd, *J.*, 1945, 660;

Atherton and Todd, *J.*, 1947, 674), however, *dibenzhydryl aminophosphonate*, *dibenzhydryl benzylaminophosphonate*, and *dibenzhydryl* cyclo*hexylaminophosphonate* were prepared in good yield. Hydrogenolysis of the last named compound showed that, as anticipated, the benzhydryl groups were more slowly removed than benzyl groups.

It is obvious that substituted diazomethanes could be employed for the preparation of organic phosphates. This method has been used, for example, by Reichstein and Schindler (*Helv. Chim. Acta*, 1940, **23**, 669), who prepared deoxycorticosterone phosphate by interaction of 21-diazoprogesterone and phosphoric acid. By using dibenzyl hydrogen phosphate in place of phosphoric acid in such reactions, not only would the protecting benzyl groups in the products be of advantage in various directions, but yields would doubtless be better, since there would be no danger of the formation of di- or tri-esters. By this means we prepared in model experiments *benzhydryl dibenzyl phosphate* and *desyl dibenzyl phosphate* from diphenyldiazomethane and phenylbenzoyldiazomethane in excellent yield; in the same way, ethyl dihydrogen phosphate was readily prepared by interaction of diazoethane with dibenzyl phosphate and hydrogenation of the initial product. The use of this method of phosphorylation is severly restricted by the inaccessibility of many substituted diazomethanes, but it may be of value in special cases.

Experimental.

Preparation of Dibenzyl Phosphite by the Use of Phenyldiazomethane.—Phenyldiazomethane(5·9 g., 2 mols.) dissolved in dry ether (100 c.c.) was gradually added (*ca.* 20 minutes) to an ice-cold stirred solution of phosphorous acid (2·05 g., 1 mol.) in dry ether (200 c.c.). After removal of ether, an attempt was made to distil the liquid residue at 10^{-2} mm.; the product began to distil at 160° but almost immediately violent decomposition occurred. Accordingly, the preparation was repeated and the product purified by low-temperature distillation as described in Part I (*loc. cit.*); dibenzyl phosphite (5·5 g., 84%), m. p. 17°, was thus obtained.

Purification of Dibenzyl Phosphite.—Crude dibenzyl phosphite (300 g.) prepared as in Part I (*loc. cit.*) is saturated with gaseous ammonia, filtered from any solid which separates, and *N*-methylmorpholine (3% by weight) added. The apparatus used for distillation is a two-necked retort with a long side arm; the two necks are fitted respectively with a thermometer and a capillary admitting nitrogen, and the side-arm is connected by a three limbed " pig " leading to the receivers. The above mixture is placed in the retort, the thermometer and capillary sealed in with polythene, and the retort immersed in an oil-bath and attached to a high-vacuum system giving a pressure of *ca.* 10^{-1} mm. *N*-Methylmorpholine distils off and is collected in a liquid-air trap, and the bath is then heated as rapidly as possible to 180—190°, at which temperature dibenzyl phosphite begins to distil; a small fore-run is usually obtained during the heating. Distillation is carried out as rapidly as possible, care being taken to keep the bath temperature uniform and to avoid local overheating. Should any sign of decomposition occur in the retort, or should distillation appear to slacken, the operation is interrupted *immediately*, the vacuum cut off, the bath removed, and the system flooded with nitrogen. With this procedure and the above quantities, pure dibenzyl phosphite (*ca.* 240 g.), b. p. 165°/10^{-1} mm. (bath temp. 180—190°), is obtained.

Action of Sulphuryl Chloride on Dialkyl Phosphites.—(a) *Diisopropyl phosphite.* The phosphite (33·2 g.) was stirred while sulphuryl chloride (28 g.) was added so as to maintain the temperature at 35—40°. The mixture was stirred for a further 90 minutes, sulphur dioxide and hydrogen chloride removed by reducing the pressure, and the product distilled through a short Vigreux column. Di*iso*propyl chlorophosphonate (33 g., 82%) distilled at 95—96°/14 mm. For characterisation, a portion (4 g.) was allowed to react with aniline; di*iso*propyl anilinophosphonate (5 g., 97%) was obtained, m. p. and mixed m. p. 120—121°.

(b) *Diethyl phosphite.* In exactly similar fashion diethyl phosphite (27·6 g.) gave diethyl chlorophosphonate (31 g., 90%), characterised by conversion into diethyl anilinophosphonate, m. p. and mixed m. p. 95—96°.

(c) *Dibenzyl phosphite.* A slow stream of nitrogen was passed through a stirred solution of dibenzyl phosphite (13·1 g.) in carbon tetrachloride (150 c.c.) while sulphuryl chloride (7 g.) in carbon tetrachloride (25 c.c.) was added during 20 minutes, the temperature being maintained at 17—19° by external cooling. Nitrogen was blown through the solution at a more rapid rate for 90 minutes, and the content of dibenzyl chlorophosphonate determined by addition of aniline (15 c.c.). On working up in the usual manner, dibenzyl anilinophosphonate (16·3 g., 92%) was isolated; recrystallised from *cyclo*hexane it had m. p. 91—92°, undepressed by an authentic specimen.

Reaction of Dibenzyl Chlorophosphonate with n*-Butanol in Presence of Pyridine.*—(a) *Low-temperature reaction.* A solution of dibenzyl chlorophosphonate (from 5·24 g. of dibenzyl phosphite) in carbon tetrachloride (20 c.c.) was added to an ice-cold mixture of *n*-butanol (1·62 g.), dry pyridine (4 c.c.), and chloroform (15 c.c.) at such a rate that the temperature did not exceed 10°. After standing overnight the mixture was washed with dilute acid, alkali, and water, dried (Na_2SO_4), and evaporated under reduced pressure. The colourless oil so obtained (6 g.) was dissolved in ethanol (100 c.c.) and hydrogenated with palladium oxide (0·1 g.) in presence of dilute sulphuric acid (1 c.c. of N); hydrogen uptake (900 c.c.) was complete in 30 minutes. Catalyst was removed by filtration, and the solution exactly neutralised (phenolphthalein) with saturated aqueous barium hydroxide and evaporated to dryness under reduced pressure. The residue was extracted with boiling water, and the extract filtered and concentrated under reduced pressure until crystallisation set in. On standing, barium *n*-butyl phosphate separated as its dihydrate (2·65 g., 56%) (Found : Ba, 42·2. Calc. for $C_4H_9O_4PBa,2H_2O$: Ba, 42·2%).

(b) *High-temperature reaction.* An exactly comparable experiment to that described in (a) was carried out, but the temperature during the addition of the dibenzyl chlorophosphonate was maintained

at 35—40° and the product worked up after 3½ hours. The yield of barium *n*-butyl phosphate (4·13 g., 63%) was appreciably higher than in (a) (Found : Ba, 42·1. Calc. for $C_4H_9O_4PBa,2H_2O$: Ba, 42·2%).

Reaction of Dibenzyl Chlorophosphonate with n-*Butanol in Presence of* N-*Methylmorpholine.*—Reactions exactly parallel to (a) and (b) above were carried out using *N*-methylmorpholine in place of pyridine.

Low-temperature reaction. A considerable quantity (3·85 g.) of crystalline solid separated and was filtered off. It proved to be *N*-benzyl-*N*-methylmorpholinium chloride, m. p. 236° (decomp.) (Found : Cl, 15·6. Calc. for $C_{12}H_{18}ONCl$: Cl, 15·6%). The liquid product obtained from the filtrate resisted hydrogenation.

High-temperature reaction. The crystalline material (3·2 g.) which separated during the reaction appeared from its properties and chlorine content (Found : Cl, 19·0%) to be a mixture of *N*-methylmorpholine hydrochloride and *N*-benzyl-*N*-methylmorpholinium chloride. The oily product again could not be hydrogenated.

Barium Ethyl Phosphate.—Ethanol (4 c.c.) and 2 : 6-lutidine (4 c.c.) were added to a solution of dibenzyl chlorophosphonate (from 5·24 g. of dibenzyl phosphite) in dry carbon tetrachloride (100 c.c.), and the mixture left at room temperature for 5 hours. The neutral oily product was isolated and hydrogenated in aqueous ethanol using a palladium oxide catalyst. Traces of chloride were removed with silver sulphate, and excess of silver precipitated with hydrogen sulphide. The filtered solution was neutralised to phenolphthalein with aqueous barium hydroxide, barium sulphate spun off, and the solution concentrated to small bulk. On addition of ethanol, barium ethyl phosphate separated as its crystalline dihydrate (3·8 g., 64%) (Found : Ba, 45·4. Calc. for $C_2H_5O_4PBa,2H_2O$: Ba, 46·2%). With pyridine in place of lutidine, the yield was 50% (Part I, *loc. cit.*). A similar experiment with *n*-butanol in place of ethanol gave barium *n*-butyl phosphate in 62% yield.

Barium Glucose-6 Phosphate.—(a) *By the use of pyridine.* Dibenzyl chlorophosphonate (from 13·1 g. of dibenzyl phosphite) in dry chloroform (50 c.c.) was added dropwise to a solution of 1 : 2-*iso*propylidene D-glucose (11 g.) in pyridine (100 c.c.) at −10° during 75 minutes. Stirring was continued while the mixture was allowed to warm to room temperature. The mixture was then set aside overnight, and evaporated under reduced pressure to a syrup, which was taken up in chloroform, washed with dilute sulphuric acid, then water, dried (Na_2SO_4), and evaporated. The residue was dissolved in ethanol, the solution refluxed for 30 minutes with Raney nickel (5 g.) to remove possible catalyst poisons, filtered, and then hydrogenated with the use of a mixture of palladium oxide (0·5 g.) and palladised charcoal (1·0 g.) as catalyst. Hydrogenation was at first rapid (70 c.c./min.), but it became slow (3 c.c./min.) after 1500 c.c. had been absorbed, and ceased after a total of 2160 c.c. hydrogen had been taken up. The *iso*propylidene group was removed from the product, and glucose-6 phosphate isolated as its barium salt (8 g., 42%; $[\alpha]_D^{20°}$ + 11·8°) by the procedure of Levene and Raymond (*J. Biol. Chem.*, 1930, **89**, 479).

(b) *By the use of* 2 : 6-*lutidine.* Dibenzyl chlorophosphonate (from 5·24 g. of dibenzyl phosphite) in chloroform (25 c.c.) was added dropwise to a stirred ice-cold solution of 1 : 2-*iso*propylidene D-glucose (4·4 g.) in 2 : 6-lutidine (100 c.c.) during 3 hours. The mixture was stirred at 0° for 4 hours and set aside overnight. Chloroform and the main bulk of lutidine were removed under reduced pressure, aqueous barium hydroxide added until the mixture was alkaline to phenolphthalein, and liberated lutidine removed by concentrating the mixture under reduced pressure to *ca.* 100 c.c. Methanol (100 c.c.) was added, together with sufficient sulphuric acid to make the acid concentration N/5, and the homogeneous solution heated on the steam-bath for 2 hours. From the resulting solution, glucose-6 phosphate was isolated as its barium salt (3·25 g.). The mother liquors from the barium salt were freed from barium by titration with sulphuric acid, using rhodizonic acid as indicator, and filtration from barium sulphate. The solution was evaporated, and the residue dissolved in aqueous ethanol (75%) and hydrogenated with the use of a palladium oxide catalyst. From the product a further quantity of barium glucose-6 phosphate (1·25 g.) was obtained. (Total yield, 4·5 g., 59%; $[\alpha]_D^{20°}$ + 12·3°.)

In a similar way barium glucose-6 phosphate was prepared from 3 : 5-benzylidene-1 : 2-*iso*propylidene D-glucose; the yield was 38% with pyridine and 49% with 2 : 6-lutidine. The preparation of glucose-6 phosphate by direct treatment of unprotected D-glucose with dibenzyl chlorophosphonate in presence of pyridine, followed by hydrogenation, was unsatisfactory, the yield being only *ca.* 15%.

Dibenzhydryl Phosphite.—A solution of phosphorous acid (13·7 g.) in dry ether (150 c.c.) was added gradually to an ice-cold solution of diphenyldiazomethane in light petroleum (prepared from 62 g. of benzophenone hydrazone according to Staudinger, Anthes, and Pfenninger, *Ber.*, 1916, **49**, 1932) until the red colour of the diazo-compound was discharged. The bulky colourless crystalline precipitate (52 g., 75% calculated on benzophenone hydrazone) was collected, washed with ether–light petroleum, and dried at room temperature in a desiccator (Found : C, 74·7; H, 5·9. $C_{26}H_{23}O_3P$ requires C, 75·4; H, 5·6%). On being heated, the product decomposed gradually above 60° and melted completely at 105°; placed in a pre-heated bath it decomposed sharply at 78°, resolidified, and ultimately melted again at 105°. Recrystallisation from hot solvents gave dibenzhydryl ether, m. p. 108—109°. The phosphite could be obtained as long felted needles by adding light petroleum to an ice-cold solution in ethyl acetate, but then showed the same behaviour on being heated as the initial product, and gave similar analytical figures.

Dibenzhydryl cyclo*Hexylaminophosphonate.*—*cyclo*Hexylamine (1·5 g., 3 mols.) was added to a solution of dibenzhydryl phosphite (2·1 g., 1 mol.) in carbon tetrachloride (10 c.c.). The mixture was set aside for 4 hours, extracted with dilute acid, washed with water, and dried (Na_2SO_4). Evaporation gave a resin (2·5 g.) which crystallised on trituration with light petroleum. Recrystallised from *cyclo*hexane, the *product* formed colourless needles, m. p. 101—102° (Found : C, 75·1; H, 6·5; N, 2·8. $C_{32}H_{34}O_3NP$ requires C, 75·1; H, 6·7; N, 2·7%).

Dibenzhydryl Benzylaminophosphonate.—Prepared in a similar way with the use of benzylamine, this *ester* formed colourless needles, m. p. 104—105°, from hexane–*cyclo*hexane (Found : C, 76·2; H, 5·9; N, 2·5. $C_{33}H_{30}O_3NP$ requires C, 78·3; H, 5·8; N, 2·7%).

Dibenzhydryl Aminophosphonate.—Prepared by passing gaseous ammonia into a solution of dibenzhydryl phosphite in carbon tetrachloride, this *ester* formed colourless needles, m. p. 145°, from *cyclo*hexane (Found : C, 72·1; H, 5·5; N, 3·1. $C_{26}H_{24}O_3NP$ requires C, 72·8; H, 5·6; N, 3·3%).

Dibenzyl Hydrogen Phosphate.—The following method is more convenient than the literature procedures (Lossen and Köhler, *Annalen*, 1891, **262**, 196; Lynen, *Ber.*, 1940, **73**, 367). A mixture of crude dibenzyl phosphite (225 g. of 80% purity), carbon tetrachloride, and aqueous pyridine (200 g. in 600 c.c. of water) was cooled to 0°, and a solution of bromine (110 g.) in carbon tetrachloride (150 c.c.) was added with stirring during 3 hours so that the temperature did not rise above 10°. Stirring was continued for a further hour, concentrated hydrochloric acid (250 c.c.) added, and the layers separated. The carbon tetrachloride layer was washed with water and then neutralised to phenolphthalein with aqueous sodium hydroxide (170 c.c. of 10%). Acidification of the neutral aqueous layer with hydrochloric acid precipitated dibenzyl hydrogen phosphate, which was extracted with chloroform (3 × 150 c.c.), the extract evaporated, and the product recrystallised from ether. Colourless prisms (100 g.) were obtained, m. p. 79—80°, undepressed in admixture with material, m. p. 79—80°, prepared by the method of Lynen (*loc. cit.*). The impurities normally present in crude dibenzyl phosphite apparently have an adverse effect, since the yield of product is higher (75%) when distilled material is employed.

Benzhydryl Dibenzyl Phosphate.—Ethereal diphenyldiazomethane was added to a suspension of dibenzyl hydrogen phosphate (4·7 g.) in ether (50 c.c.) until a permanent pink colour was produced. After addition of more dibenzyl hydrogen phosphate (0·1 g.) to discharge the colour, the solution was washed with aqueous sodium hydroxide, dried, and evaporated. On adding a little light petroleum and allowing to stand overnight in the ice-chest, the oily product solidified. Recrystallised from light petroleum, the *ester* had m. p. 72—72·5° (Found: C, 73·1; H, 5·6. $C_{27}H_{25}O_4P$ requires C, 73·0; H, 5·6%). The yield was almost theoretical.

Desyl Dibenzyl Phosphate.—Phenylbenzoyldiazomethane (4·44 g., 1 mol.) was added to a solution of dibenzyl hydrogen phosphate (5·56 g., 1 mol.) in benzene (50 c.c.). After 48 hours the pale yellow solution was washed with aqueous sodium hydroxide, dried, and evaporated. The residual oil crystallised on standing with light petroleum and then had m. p. 53—54° (yield, 7·6 g.) (Found: C, 71·1; H, 5·8. $C_{28}H_{25}O_5P$ requires C, 71·2; H, 5·4%). Attempts to purify the *ester* by further recrystallisation led to decomposition with production of oily material.

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221. *The Coupling Reaction of Cinnamyl Halides.* meso-αβ-*Divinyldibenzyl and the Phenylvinylmethyl Radical.*

By H. P. KOCH.

The composition of the isomeric diphenylhexa-1 : 5-diene mixture produced by the coupling of cinnamyl halides over magnesium has been determined spectrographically, and the course of the reaction is discussed with reference to existing work on crotylmagnesium halides. Pure 1 : 6-diphenylhexa-1 : 5-diene free from fluorescent impurities has been obtained, and meso-3 : 4-*diphenylhexa*-1 : 5-*diene* has been isolated and characterised for the first time. The new hydrocarbon undergoes thermal rearrangement to a mixture of 1 : 6- and 1 : 4-diphenylhexa-1 : 5-dienes above 100°, largely or entirely by a mechanism involving the intermediate formation of free phenylvinylmethyl radicals. Phenylvinylmethyl is also produced transitorily when allylbenzene reacts with diacetyl peroxide, or by dissociation of 1 : 4-diphenylhexa-1 : 5-diene above 200°. Alder's conclusions relating to the course of addition of maleic anhydride or azodicarboxylic ester to allylbenzene are re-examined in the light of the present work.

THE coupling of cinnamyl halides over metallic magnesium in ether was first studied by Rupe and Bürgin (*Ber.*, 1910, **43**, 172) who thereby prepared crystalline dicinnamyl (1 : 6-diphenylhexa-1 : 5-diene) (I; R = Ph) accompanied by a second incorrectly formulated oily hydrocarbon which was much later identified by Prévost (*Bull. Soc. chim.*, 1931, **49**, 1372) and by Gilman and Harris (*J. Amer. Chem. Soc.*, 1932, **54**, 2072) as the isomeric 1 : 4-diphenylhexa-1 : 5-diene, also designated *iso*dicinnamyl (II; R = Ph). Contrary to statements made by the original authors, it was claimed by von Braun and Köhler (*Ber.*, 1918, **51**, 88) that cinnamyl bromide gave much better yields of the desired crystalline isomer than did the chloride, but this experience was not confirmed either by Kuhn and Winterstein (*Helv. Chim. Acta*, 1928, **11**, 87) or by the work now described. Dicinnamyl free from isomers was, however, prepared from 1 : 6-dihydroxy-1 : 6-diphenylhexane by Borsche and Wollemann (*Ber.*, 1912, **45**, 3713) and later by Schmidt and Grosser (*ibid.*, 1942, **75**, 826), although this alternative mode of synthesis does not appear to offer any advantages from the preparative point of view.

It recently became necessary to obtain a quantity of very pure dicinnamyl for spectroscopic and crystallographic examination in this laboratory, and the detection of a hitherto unsuspected trace impurity in the hydrocarbon, as well as the isolation of an interesting new crystalline isomer, led to a re-investigation of all aspects involved in the coupling reaction of cinnamyl halides.

Dicinnamyl.—In the work of all the authors cited above, dicinnamyl is described as a crystalline solid, m. p. 81°, characterised by a strong bluish or violet fluorescence. It is now found that the optical peculiarity is caused by the presence of a trace impurity, doubtless 1 : 6-diphenylhexatriene which is known to fluoresce strongly in dilute solution (Kuhn and Winterstein, *loc. cit.*) and to exhibit an intense absorption band at 3490 A. (Hausser, Kuhn, and Smakula, *Z. physikal. Chem.*, 1935, *B*, **29**, 384). Fluorescing samples of dicinnamyl all display selective absorption near 3500 A. with varying extinction coefficients corresponding to fractional percentages of diphenylhexatriene. Both absorption and fluorescence disappear on chromatographic purification, whereby pure white dicinnamyl of greatly improved crystallising properties is obtained (cf. Experimental). The hydrocarbon may also be prepared almost or entirely pure if it is isolated from the isomeric diphenylhexadiene mixture at the lowest possible temperature (distillation bath below 150°) in a high-vacuum still, and since pure dicinnamyl acquires fluorescence through heating in a vacuum at 250°, the trace impurity appears to be due to slight dehydrogenation taking place at elevated temperatures. Kuhn and Winterstein (*loc. cit.*) drew attention to the great stability and pronounced aromatic character of 1 : 6-diphenylhexatriene, the ready appearance of which in samples of dicinnamyl is paralleled by the formation of traces of stilbene whenever dibenzyl is prepared (Ramart-Lucas and Hoch, *Compt. rend.*, 1931, **192**, 53; Ley and Dirking, *Ber.*, 1934, **67**, 1331). The thermal dehydrogenation also finds an analogy in the behaviour of *cyclo*hexene, which gives rise to spectroscopically detectable amounts of *cyclo*hexadiene on simple distillation (Stücklen, Thayer, and Willis, *J. Amer. Chem. Soc.*, 1940, **62**, 1717; cf. Bateman and Koch, *J.*, 1944, 600).

Rather unexpectedly, it was not found possible to convert dicinnamyl into diphenylhexatriene by the action of *N*-bromosuccinimide under the conditions employed by Karrer and his collaborators, who thereby succeeded in dehydrogenating the 2 : 6-ethylenic end-groups of lycopene to the 2 : 4 : 6-unsaturated fully conjugated polyene structure (*Helv. Chim. Acta*, 1945, **28**, 793; 1946, **29**, 573). Dicinnamyl did not react with *N*-bromosuccinimide even after the addition of benzoyl peroxide, for reasons which are not understood. Both 1-phenylpropylene (Ziegler *et al.*, *Annalen*, 1942, **555**, 80) and diallyl (Karrer *et al.*, *loc. cit.*, 1946) form bromo-derivatives by substitution under these conditions.

No other modification of dicinnamyl is known to exist, and there cannot be any doubt (see this vol., p. 1123) that the hydrocarbon of m. p. 81° possesses the *trans-trans*-configuration.

meso-αβ-*Divinyldibenzyl.*—When the hydrocarbon mixture resulting from the coupling of cinnamyl halides was distilled in a high vacuum, the initial fractions deposited a new crystalline isomer, $C_{18}H_{18}$, of m. p. 87°. This hydrocarbon was found to be doubly unsaturated, giving rise to a *tetrabromide*, and its ultra-violet absorption spectrum, very similar to that of dibenzyl or allylbenzene, indicated that the two double bonds were out of conjugation with the benzene rings. The new isomer must therefore be formulated as **3 : 4-*diphenyl*-1 : 5-*hexadiene*** (**αβ-*divinyldibenzyl***) (III; R = Ph) which can theoretically exist in either the racemic DL- or the internally compensated *meso*-form. A determination of the crystallographic space-group (this vol., p. 1118) proved that the molecules were centrosymmetric and therefore necessarily of the *meso*-configuration. This structure was further confirmed by an isomorphous relationship with the *meso*-αβ-diethyldibenzyl examined by Carlisle and Crowfoot (*J.*, 1941, 6). The latter hydrocarbon has almost identical unit-cell dimensions, and a specimen failed to depress the slightly lower m. p. of the former on mixing. Through the courtesy of Professor E. C. Dodds it was also established that *meso*-αβ-divinyldibenzyl displayed weak oestrogenic activity comparable with that of *meso*-αβ-diethyldibenzyl, the hydrocarbon analogue of hexoestrol (this vol., p. 1116).

Since even the most carefully isolated αβ-divinyldibenzyl fractions always contained small amounts of an oily admixture, there appears to be evidence that the racemic DL-form was also produced during the coupling reaction. This stereoisomer would not be expected to crystallise, by analogy with DL-αβ-diethyldibenzyl which has been described as a liquid (cf. Carlisle and Crowfoot, *loc. cit.*). According to Liepins (*Chem. Abs.*, 1931, **25**, 3328), the coupling of phenylalkylmethyl halides over magnesium generally results in the formation of both DL- and *meso*-forms, although the respective rates may be different.

meso-αβ-Divinyldibenzyl undergoes rapid rearrangement when attempts are made to distil the hydrocarbon in a low vacuum. This phenomenon is no doubt responsible for the failure of previous authors to isolate it (cf. Gilman and Harris, *loc. cit.*) and will be further discussed below.

The Course of the Coupling Reaction.—Although the quantitative separation of αβ-divinyldibenzyl and dicinnamyl from the oily *iso*dicinnamyl (II; R = Ph) present in the coupled hydrocarbon mixture is impossible to achieve, the three isomers may be estimated in the product

by ultra-violet absorption analysis of the fractions obtained on high-vacuum distillation. This method is based on the wide differences in maximum extinction near 2500 A. associated

$$\underset{\text{(I.)}}{CHR{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CHR} \qquad \underset{\text{(II.)}}{CHR{:}CH{\cdot}CH_2{\cdot}CHR{\cdot}CH{:}CH_2} \qquad \underset{\text{(III.)}}{CH_2{:}CH{\cdot}CHR{\cdot}CHR{\cdot}CH{:}CH_2}$$

with the presence of 0, 1, or 2 conjugated styrene systems in the three hydrocarbons (ε = 370, 20,000, and 33,000, respectively). The result of such an analysis proves to be very similar to the composition of the analogous mixture produced by the coupling of crotyl halides which has been studied by Young, Roberts, and Wax (*J. Amer. Chem. Soc.*, **1945**, **67**, 841). The relevant data are given in the table below, where the figures in parentheses refer to the amounts of crystalline material which can actually be iolated in a pure state.

Coupling halide, CHR:CH·CH$_2$X.	Isomers, %. (I).	(II.)	(III).	Total hydrocarbon yield, %.
Cinnamyl chloride (R = Ph, X = Cl)	20 (11)	75	3—5 (0·85)	85
Cinnamyl bromide (R = Ph, X = Br)	— (10)	—	— (1)	—
Crotyl chloride (R = Me, X = Cl)	12	82	6	72
Crotyl bromide (R = Me, X = Br)	10	88	2	—

The overall course of either coupling reaction may be formulated as

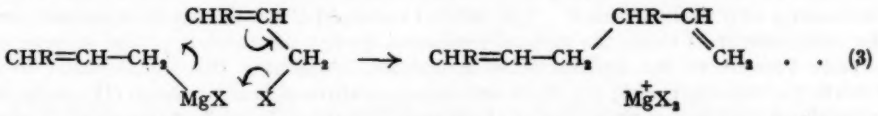
$$CHR{:}CH{\cdot}CH_2X + Mg \longrightarrow \left\{\begin{array}{c} CHR{:}CH{\cdot}CH_2{\cdot}MgX\ (a) \\ \text{and/or} \\ CHR(MgX){\cdot}CH{:}CH_2\ (b) \end{array}\right\} \quad . \ . \ . \ . \ (1)$$

followed by

$$\left\{\begin{array}{c} CHR{:}CH{\cdot}CH_2{\cdot}MgX\ (a) \\ \text{and/or} \\ CHR(MgX){\cdot}CH{:}CH_2\ (b) \end{array}\right\} + CHR{:}CH{\cdot}CH_2X \longrightarrow \text{(I), (II), and (III)} + MgX_2 \quad . \ . \ (2)$$

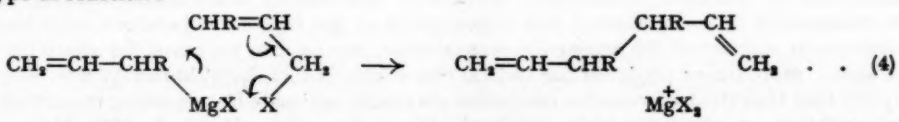
The table shows that, for both cinnamyl and crotyl halides, the unsymmetrical *iso*-structure (II) is the main constituent of the product, which may therefore be regarded as formally derived from the " abnormal " secondary form (*b*) of the Grignard reagent. The yield of divinyl compound (III) is small in either case, but the proportion of the " normal " product (I) is significantly higher for cinnamyl than for crotyl halides. This difference may well be due to additional resonance stabilisation of (*a*) or (I) arising from conjugation in the cinnamyl residue. The percentages of dicinnamyl (I) and *iso*dicinnamyl (II) formed also correspond with the yields of 1-propenylbenzene (25%) and allylbenzene (75%) produced on hydrolysis of the Grignard reagent from cinnamyl halides (Young *et al.*, *ibid.*, 1939, **61**, 12; 1947, **69**, 688).

Recently, Young and Roberts (*ibid.*, 1946, **68**, 1472) have advanced strong arguments to support the view that the Grignard reagent from crotyl halides actually exists predominantly in the *primary* form (*a*), conclusions which might be expected to apply *a fortiori* to the resonance-stabilised cinnamyl compounds. On this basis, the preferential formation of (II) over (I) would appear to involve a bimolecular cyclic mechanism (cf. Gilman, " Organic Chemistry ", 2nd ed., 1943, p. 1880) as a facile alternative to the conventional " normal " mode of reaction :



(3)

The small yield of αβ-divinyldibenzyl may be regarded as evidence *either* that resonating organic ions or radicals also appear during the coupling reaction *or* that at least a fraction of the Grignard reagent exists in the " abnormal " form (*b*), giving rise to the new isomer by a similar cyclic type of reaction :

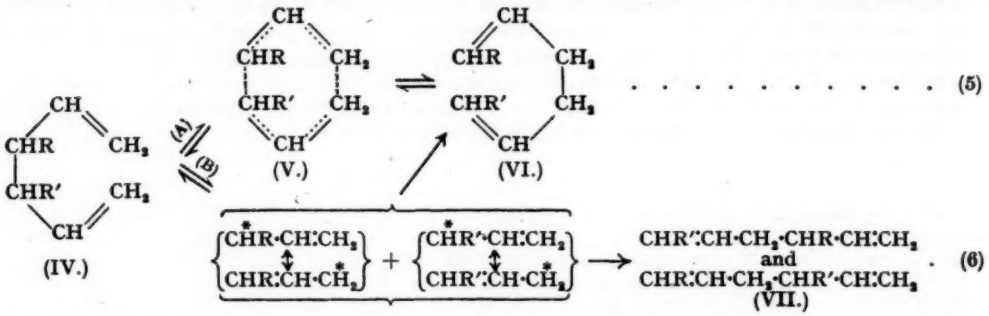


(4)

There is no reason to believe that the cinnamyl halides themselves may be partly isomerised to the secondary phenylvinylmethyl halides under the conditions of the synthesis.

Thermal Rearrangement of meso-αβ-*Divinyldibenzyl.*—The new crystalline isomer (IV; R = R′ = Ph) is stable at 100°, but on heating to 150° it is readily and quantitatively rearranged

to an approximately 3 : 2 mixture of dicinnamyl and *iso*dicinnamyl. Two competing processes may be formulated to account for the initial step in the rearrangement of substituted hexa-1 : 5-dienes generally: an electron shift (A) giving rise to a cyclic transition complex (V), or dissociation (B) into free radicals which are stabilised by resonance involving two different positions of the unshared electron. Both mechanisms are unimolecular but distinguished by



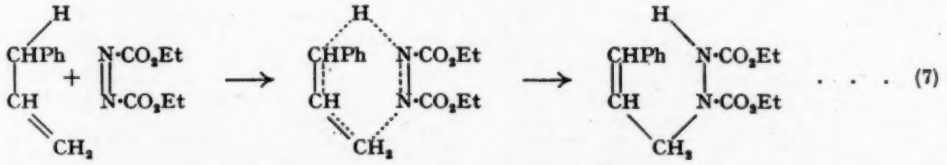
the fact that (A) cannot give more than one new isomer, whereas (B) is expected to yield two or three different products of radical recombination, depending on whether or not R = R′. Levy and Cope (*J. Amer. Chem. Soc.*, 1944, **66**, 1684) have established the general validity of the intramolecular cyclic scheme (A) by showing that (IV) could be incompletely and reversibly rearranged to pure (VI) when R = H and R′ = Me, by heating to 300°. Similar isomerisation to a single product occurred when R = Ph and R′ = Me, but in this case the reaction proceeded at a much lower temperature (165—185°) and rearrangement was complete and irreversible (*loc. cit.*), features which might be expected from the electronic activating effect of the phenyl substituent and the conjugated nature of the product. In the present work on αβ-divinyldibenzyl (IV; R = R′ = Ph), isomerisation was even more facile but the presence of *iso*dicinnamyl (VII) in the product indicates that the alternative process (B) must be operative at least in part. The further activating effect of the second phenyl group evidently assists the radical dissociation relatively more than the formation of the hypothetical cyclic transition complex. A rough calculation of the bond energies involved bears out the theoretical soundness of the free-radical reaction scheme. According to Orr (quoted by Bolland and Gee, *Trans. Faraday Soc.*, 1946, **42**, 244), the resonance energy of the divinylmethyl radical amounts to 30 kcals., and allowing for the small difference between the values recorded for allyl and benzyl radicals, the resonance energy of phenylvinylmethyl might be expected to be about 3 kcals. less than this, *i.e.*, near 27 kcals. The central bond of αβ-divinyldibenzyl would then be weakened by twice this amount below the normal single bond strength of 81 kcals. The resultant theoretical bond strength of approximately $81 - 2 \times 27 = 27$ kcals. is in good accord with the experimentally required isomerisation temperature. The resonance energy of the phenylvinylmethyl radical may therefore be regarded as the main driving force for a radical dissociation ultimately leading to the formation of the more stable molecular species of dicinnamyl and *iso*dicinnamyl. The central bonds of these two isomers are not weakened by the full amount of twice the radical resonance energy on account of the styrene conjugation already present in the undissociated molecules. Assuming this stabilisation to amount to 7 kcals. per styrene group, the dissociation temperature of *iso*dicinnamyl (II) might be expected to be about 100° higher than that of αβ-divinyldibenzyl (III), while dicinnamyl (I) should still be stable at 250°. This prediction was verified by experiment: (I) and (II) were unaffected by temperatures near 200°, but at 250° only dicinnamyl (I) remained unchanged while *iso*dicinnamyl (II) was largely transformed into a polymeric gum from which some dicinnamyl could be separated by vacuum distillation. Evidently free-radical dissociation leading to partial rearrangement of *iso*dicinnamyl had taken place at the higher temperature, affording indirect evidence in support of the analogous isomerisation mechanism proposed for αβ-divinyldibenzyl at 150°. More direct proof for the radical dissociation of αβ-divinyldibenzyl was also obtained by the fact that the hydrocarbon resembled dibenzoyl peroxide in catalysing the initial stages of autoxidation of ethyl linoleate (Bolland, *Proc. Roy. Soc.*, 1946, *A*, **186**, 218). In these experiments, divinyldibenzyl appeared to decompose by a heterogeneous process, with an activation energy in the neighbourhood of 30 kcals.

Attempts to shift the double bonds of αβ-divinyldibenzyl into conjugation to furnish 3 : 4-diphenylhexa-2 : 4-diene by treatment with hot alcoholic potassium hydroxide proved

unsuccessful. No conjugative isomerisation could be observed before the competing thermal rearrangement removed the unconjugated isomer from the sphere of influence of the alkali.

Coupling of Phenylvinylmethyl Radicals.—In the thermal rearrangement of αβ-divinyldibenzyl a 67% yield of *iso*dicinnamyl and 33% of dicinnamyl might be expected if there were strict equivalence between the two alternative canonical forms of the coupling radicals. Since the relative yields of dicinnamyl are markedly higher than this (~ 60%), it must be concluded either that the cinnamyl form is preferentially stabilised by conjugation or that some proportion of the dicinnamyl in the product arises from the non-dissociative intramolecular cyclic reaction (5) referred to above. Phenylvinylmethyl radicals were also prepared directly by decomposition of diacetyl peroxide in allylbenzene (cf. Kharasch, McBay, and Urry, *J. Org. Chem.*, 1945, **10**, 401), and on dimerisation they did produce *iso*dicinnamyl in excess of dicinnamyl, but the exact relative proportions of recombined phenylvinylmethyl and cinnamyl residues resulting from this reaction could not be ascertained on account of the formation of a considerable polymeric fraction of unknown composition. No trace of divinyldibenzyl could be isolated from the product, either on account of the high temperature required for the peroxide decomposition (near 120°) or because of preferential further attack by the reagent at the tertiary C–H group of the divinyldibenzyl, resulting in hydrocarbons of greater complexity which may form part of the polymeric fraction (cf. related observations of Kharasch *et al.*, *loc. cit.*). The formation of the diphenylhexadienes from allylbenzene and diacetyl peroxide offered no preparative advantages over the coupling of cinnamyl halides, and attempts to couple phenylvinylmethylsodium (sodio-allylbenzene) by the action of iodine proved completely abortive.

The intermediate appearance of phenylvinylmethyl radicals has been postulated by Alder, Pascher, and Schmitz (*Ber.*, 1943, **76**, 27) in order to explain the course of addition of maleic anhydride (at 200°) or azodicarboxylic ester (at room temperature) to allylbenzene. In either case the *only* isolable addition product was derived from the rearranged cinnamyl and not from the original phenylvinylmethyl residue of allylbenzene, and the double-bond shift was held to be due to primary dissociation of a C–H bond followed by conjugative isomerisation of the phenylvinylmethyl radical before combination with the addendum. Similar free-radical mechanisms were invoked to account for the products from hexa-1 : 5-diene for which again *exclusively* abnormal addition arising from double-bond shift was observed although no conjugation occurred here as a result of the rearrangement. Since it has been shown in the present work that the coupling of phenylvinylmethyl radicals invariably produces *iso*dicinnamyl as well as dicinnamyl, the mechanistic conclusions of Alder and his co-workers would not seem to be easily compatible with the dual reactivity of the phenylvinylmethyl radical. In the absence of special factors, the proposed scheme of addition of maleic anhydride or azodicarboxylic ester to allylbenzene should be expected to furnish a significant porportion of *normal* as well as abnormal products, just as the " abnormal " double-bond shift is incomplete during the addition of molecular oxygen to the (substituted) divinylmethyl radicals involved in ethyl linoleate oxidation (Bolland and Koch, *J.*, 1945, 445). If indeed Alder's observations on the quantitative completeness of the rearrangement-addition are well founded experimentally, they could equally well be accounted for by an intermolecular cyclic reaction mechanism analogous to the Diels–Alder *diene* addition and also somewhat reminiscent of the intramolecular rearrangements of type (5) :



. . . (7)

Experimental.

Diphenylhexa-1 : 5-dienes from Cinnamyl Chloride.—Dry hydrogen chloride was bubbled through melted *trans*-cinnamyl alcohol with ice-cooling to give cinnamyl chloride (80%), b. p. 106—112°/12 mm. (nitrogen), m. p. 8°. A stirred suspension of magnesium turnings (9 g.) in dry ether (50 c.c.) was activated by the addition of a few drops of methylmagnesium iodide solution, and cinnamyl chloride (111 g.) in dry ether (250 c.c.) was then added dropwise at such a rate that the mixture was kept spontaneously refluxing. After further heating on the water-bath for 30 mins., the contents of the flask were poured on ice and ammonium chloride, and the product obtained as a partly crystallised light yellow oil after working up in the usual manner. Slow distillation through a short Vigreux column at 10^{-3} mm. gave a small forerun, followed by ten separately collected fractions boiling between 80° and

135°, a final fraction being recovered from the residue in the distilling flask (total yield of hydrocarbon 72 g. = 85%; cf. Gilman and Harris, *loc. cit.*, 75%).

The small initial fractions (bath temp. 135—140°) soon deposited crystals of *meso*-$\alpha\beta$-divinyldibenzyl, while the higher fractions contained increasing amounts of solid dicinnamyl. Each fraction was weighed, and its approximate composition analysed by determining the maximum light absorption intensity in the region of 2500—2550 A., it being assumed that the first fractions consisted of the 3 : 4- and the 1 : 4-diphenyl-substituted hexa-1 : 5-dienes only, while the higher fractions were treated as mixtures of the 1 : 4- with the 1 : 6-isomer (cf. spectral data on pure compounds given below). The total hydrocarbon was accordingly estimated to contain about 3% of 3 : 4-diphenylhexa-1 : 5-diene ($\alpha\beta$-divinyldibenzyl) and 20% of 1 : 6-diphenylhexa-1 : 5-diene (dicinnamyl) after high-vacuum distillation. The amount of $\alpha\beta$-divinyldibenzyl actually formed during the coupling reaction may well have been somewhat greater than that collected in the distillate, on account of its thermal instability. The proportion of 1 : 4-diphenylhexa-1 : 5-diene (*iso*dicinnamyl) present in the reaction product is then estimated by difference to be about 75%.

By crystallisation from ethanol of the first semi-solid fractions, *meso*-$\alpha\beta$-divinyldibenzyl could be isolated in almost pure condition in a yield of 680 mg. (0·85% of the theoretical based on the cinnamyl chloride), and the higher fractions, after one redistillation, similarly gave 9·5 g. (11%) of crystalline *trans*-dicinnamyl (Gilman and Harris, *loc. cit.*, obtained 8·75%; Kuhn and Winterstein, *loc. cit.*, 10%).

meso-$\alpha\beta$-*Divinyldibenzyl* (3 : 4-*Diphenylhexa*-1 : 5-*diene*).—This *compound* formed small white monoclinic crystals from ethanol or by high-vacuum sublimation, m. p. 87—87·5° (Found: C, 92·3; H, 7·9. $C_{18}H_{18}$ requires C, 92·26; H, 7·74%). For crystallographic data, see this vol., p. 1118. A sample of *meso*-$\alpha\beta$-diethyldibenzyl kindly supplied by Dr. Crowfoot melted at 89·5—90° and did not depress the m. p. of the divinyl analogue. Light absorption in *n*-hexane: Max., 2600 A., $\epsilon = 370$ (stilbene-free dibenzyl has max., 2600 A., $\epsilon = 420$. For absorption of allylbenzene, cf. below). *Tetrabromide*: Microcrystalline powder from acetone, m. p. 241° (Found: Br, 57·6. $C_{18}H_{18}Br_4$ requires Br, 57·7%). No colour change or crystalline adduct was obtained on mixing either *meso*-$\alpha\beta$-divinyldibenzyl or *meso*-$\alpha\beta$-diethyldibenzyl with 2 mols. of *s*-trinitrobenzene in a small volume of chloroform, conditions under which stilbene-free dibenzyl forms a rather unstable colourless complex of m. p. 103° (cf. Sudborough, *J.*, 1916, **109**, 1339). *Oestrogenic assay* (data supplied by Professor Dodds): the total dose of *meso*-$\alpha\beta$-divinyldibenzyl contained in 3 c.c. of sesame oil solution was given as six twice-daily injections of 0·5 c.c. each in spayed rats. Smears consisting entirely of cornified and/or epithelial cells were taken to indicate positive response. The E.D. 50 for the oestrogenic activity of the hydrocarbon by this technique was found to lie between 1 and 10 mg.

trans-*Dicinnamyl*.—This formed thin, irregular plates from ethyl or butyl alcohol, ethyl acetate, acetic acid, or light petroleum, b. p. 115°/0·005 mm.; m. p. 80·5—81° (lit., 81°), depressed to 75—77·5° on admixture with *meso*-$\alpha\beta$-divinyldibenzyl (Found: C, 92·3; H, 7·85. Calc. for $C_{18}H_{18}$: C, 92·26; H, 7·74%). Light absorption in *n*-hexane: Max., 2550 A., $\epsilon = 33{,}000$. Samples previously subjected to elevated reaction or distillation temperatures exhibit an absorption band near 3500 A. with ϵ values ranging up to 200, and a bluish or violet fluorescence in ordinary or ultra-violet light (cf. Rupe and Bürgin, *loc. cit.*; Schmidt and Grosser, *loc. cit.*). A sample obtained by distilling the diphenylhexadiene mixture from the coupling of cinnamyl chloride at the lowest possible temperature (wide-neck distilling flask with low side-arm attachment, bath at 150°, b. p. 105°/0·0005 mm.) was, however, free from the long-wave absorption and fluorescence characteristic of very dilute solutions of 1 : 6-diphenylhexatriene [cf. Kuhn and Winterstein, *loc. cit.*; Hausser, Kuhn, and Smakula (*loc. cit.*) found max. light absorption of diphenylhexatriene in ethanol at 3490 A., $\epsilon = 67{,}000$]. The trace impurity could also be removed by chromatographic adsorption from light petroleum (b. p. 40—60°) on alumina (Savory and Moore), on which dicinnamyl was only lightly held while the triene formed a rather broad band whose slow progress down the column could be conveniently followed by examination with ultra-violet light (fluorescence).

Single crystals of triene-free *trans*-dicinnamyl could be obtained from concentrated acetone solution as flat, transparent, monoclinic plates, characterised by a unit cell of dimensions $a = 15{\cdot}37$, $b = 7{\cdot}48$, $c = 6{\cdot}22$ A., $\beta = 103°$, containing two centrosymmetric molecules and having space-group symmetry of $P\,2_1/c$. The pure hydrocarbon forms an addition compound with 2 mols. of *s*-trinitrobenzene (needles, m. p. 145°, from chloroform) which is yellow with a greenish tinge, and not "light brownish-yellow" as described by Kuhn and Winterstein (*loc. cit.*) presumably because of contamination with traces of the intensely red complex formed by 1 : 6-diphenylhexatriene.

iso*Dicinnamyl* (1 : 4-*Diphenylhexa*-1 : 5-*diene*).—This isomer was obtained pure as a water-white liquid entirely devoid of fluorescence by threefold refractionation of the oily middle fractions of the diphenylhexadiene distillate; b. p. 100°/0·005 mm., $n_D^{13°}$ 1·5904 (cf. Gilman and Harris, *loc. cit.*, $n_D^{20°}$ 1·5890), light absorption in *n*-hexane, max., 2510 A., $\epsilon = 20{,}000$. A yellow colour but no compound crystals were obtained with *s*-trinitrobenzene in chloroform solution, and the addition of bromine resulted in formation of a gum. The hydrocarbon itself set to a glass on cooling.

Diphenylhexa-1 : 5-*dienes from Cinnamyl Bromide*.—Cinnamyl bromide (80 g.; b. p. 55—60°/0·02 mm.; m. p. 31°), prepared by refluxing cinnamyl alcohol with three parts of hydrobromic acid (d 1·48) for ½ hour, was brought into reaction with magnesium (5 g.) exactly as indicated under the chloride. *meso*-$\alpha\beta$-Divinyldibenzyl and dicinnamyl could be isolated by crystallisation from ethanol in yields of 550 mg. (1%) and 5·0 g. (10%), respectively.

Diphenylhexa-1 : 5-*dienes from Allylbenzene*.—Allylbenzene (b. p. 50·5—51°/17 mm.; $n_D^{15°}$ 1·5130; light absorption in *n*-hexane, max., 2600 A., $\epsilon = 210$) was prepared by reaction of phenylmagnesium bromide with allyl bromide and purified by a preliminary distillation from molten sodium, followed by rectification under reduced pressure in a Fenske–Whitmore apparatus.

(*a*) Sodio-allylbenzene was prepared in liquid ammonia solution by the method of Levy and Cope (*loc. cit.*), and the ammonia gradually replaced by dry ether and benzene. Rather less than half the theoretical amount of iodine required for a coupling reaction was absorbed fairly rapidly at room temperature and some salt was precipitated, but after addition of all the halogen, warming on the

water-bath, and working up in the usual manner, only an unpleasant dark tar was obtained which was not further investigated.

(*b*) (cf. Kharasch, McBay, and Urry, *loc. cit.*). Sodium peroxide (20 g.) was weighed into a conical flask under dry ether (200 c.c.), acetic anhydride (40 g.) was added, and the whole cooled to − 15° in a freezing mixture. Ice (*ca.* 70 g.) was then added, with vigorous shaking, in traces at first and then more rapidly during 10—15 mins. After another 10 mins.' shaking in the cold, the ethereal layer was separated, dried ($CaCl_2$), and cooled to − 78° overnight, whereupon diacetyl peroxide crystallised out in long needles. The bulk of the ether was decanted from the crystals and the remainder evaporated at − 78° into a liquid-air trap by pumping on the high vacuum for 24 hours. The cold peroxide was then covered with allylbenzene (50 g.) and allowed to warm to room temperature and dissolve. This solution was added during 1½ hours below the surface to a further quantity of allylbenzene (30 g.) kept at 120° in a 500-c.c. flask. A regular stream of gas was evolved. The product was freed from low-boiling material and excess allylbenzene (52 g.) at the water-pump, and then subjected to careful distillation in a high vacuum. After a small unidentified forerun (0·5 g.), two roughly equal fractions of diphenylhexa-1 : 5-diene (total, 11 g.) were collected and a considerable polymeric residue remained in the flask. The first fraction (b. p. 110°/10^{-3} mm., $n_D^{14°}$ 1·58) consisted mainly of *iso*dicinnamyl and did not crystallise on cooling, while the second fraction (b. p. 115—140°/10^{-3} mm.) showed distinct fluorescence and soon began to deposit crystals of dicinnamyl which could be isolated in the usual manner after further fractionation. No trace of crystalline *meso*-αβ-divinyldibenzyl was detectable in the low-boiling fractions.

Thermal Rearrangement of meso-*αβ-Divinylbenzyl.*—(*a*) On attempting to distil *meso*-αβ-divinyldibenzyl at the water-pump, only a trace of solid sublimed over, followed by an oil which soon deposited crystals of dicinnamyl. (*b*) After the substance had been heated in a sealed tube under nitrogen for 2½ hours at 100°, it re-solidified almost unchanged (m. p. 84—86°). (*c*) When heated at either 150° or 200° under nitrogen for 2½ hours, *meso*-αβ-divinyldibenzyl produced a mixture of diphenylhexadienes of intense light-absorption characteristics (max. near 2500 A., ϵ = 27,000), and much dicinnamyl crystallised out soon after cooling. (*d*) A sample (500 mg.) of the crystalline hydrocarbon was heated at 200° in an atmosphere of nitrogen for 1 hour, and the product distilled at the water-pump. Four fractions of regularly increasing b. p., solidity after cooling, fluorescence, and light-absorption intensities (ϵ = 25,000—30,000 near 2550 A.) were collected.

Thermal Rearrangement of iso*Dicinnamyl.*—(*a*) When *iso*dicinnamyl was heated for 1 hour at 200° under nitrogen, no solid was produced, and the ultra-violet absorption characteristics remained unchanged. (*b*) On heating a sample of pure *iso*dicinnamyl (1·7 g.), sealed in a vacuum, at 250—260° for 3 hours, there resulted a yellow gum with a distinct greenish fluorescence. High-vacuum distillation of this product furnished (i) a colourless oil (400 mg.) at 105—130° which was mainly unchanged *iso*dicinnamyl but exhibited the typical bluish-violet fluorescence associated with the straight-chain isomer, and (ii) a further fraction (100 mg.) at 130—150° which largely crystallised and contained dicinnamyl, identified by its crystal form and fluorescence. An undistillable polymeric residue remained in the flask.

Thermal Treatment of Dicinnamyl.—Triene-free (non-fluorescent) dicinnamyl was sealed up in a high vacuum and heated for 3 hours at 250—260°. On cooling, the hydrocarbon recrystallised unchanged except for a distinct bluish-violet fluorescence.

Isomerisation of αβ-Divinyldibenzyl in the Presence of Alkali.—(*a*) *meso*-αβ-Divinyldibenzyl (50 mg.) was heated in a sealed tube with 10% ethyl-alcoholic potassium hydroxide solution (5 c.c.) overnight. After neutralisation with dilute acid, crystals of unchanged starting material (identified by m. p. and mixed m. p.) were isolated *via* ether. (*b*) The crystalline hydrocarbon (100 mg.) was sealed up with 10% ethyl-alcoholic potassium hydroxide solution (10 c.c.) and heated to 100°. The temperature was then gradually raised to 160° during 1 hour, and kept at this level for a further hour. On working up, the product was obtained as an almost colourless oil which largely crystallised in the cold. The crystals were identified as dicinnamyl by conversion into the addition compound with *s*-trinitrobenzene (m. p. and mixed m. p.).

Attempted Reaction of Dicinnamyl with N-*Bromosuccinimide* (cf. Karrer *et al.*, *loc. cit.*).—Dicinnamyl (1·4 g.; 0·006 mol.), *N*-bromosuccinimide (0·89 g.; 0·005 mol.), and recrystallised benzoyl peroxide (5 mg.) were refluxed together in dry carbon tetrachloride (2 c.c.) for 2 hours. The filtrate was evaporated and yielded unchanged dicinnamyl (1·0 g.) after one crystallisation from ethanol.

The author would like to record his sincere gratitude to Dr. J. L. Bolland, Professor E. C. Dodds, F.R.S., and Dr. G. A. Jeffrey, through whose kind co-operation the kinetic, biological, and crystallographic measurements on *meso*-αβ-divinyldibenzyl were made available, and to Drs. L. Bateman and G. Gee for much useful discussion and criticism. Thanks are also due to Dr. W. T. Chambers for the micro-analyses reported in this paper which is based on work forming part of a programme of fundamental research undertaken by the Board of the British Rubber Producers' Research Association.

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222. *Molecular Configuration and Isomorphism in the* meso-αβ-*Divinyldibenzyl Series.*

By G. A. Jeffrey, H. P. Koch, and S. C. Nyburg.

X-Ray crystallographic studies of molecular symmetry have been combined with a consideration of scale models, ultra-violet absorption data, and the steric requirements of resonance theory in order to determine details of molecular configuration in the *meso*-αβ-divinyldibenzyl series. αβ-Diethylidenedibenzyl of m. p. 101° is shown to be the *trans-trans*-isomer of 3 : 4-diphenylhexa-2 : 4-diene. The *meso*-αβ-divinyldibenzyl, *meso*-αβ-diethyldibenzyl, αβ-diethylidenedibenzyl, and *trans*-αβ-diethylstilbene structures are all centrosymmetrical, with parallel non-coplanar aromatic ring planes. The isomorphous relationships in this series are discussed with reference to crystal geometry, miscibility, and physiological activity, and some comments are made on the spatial conformation of the corresponding phenolic molecules which belong to the class of the highly active synthetic oestrogens.

In the foregoing paper an account has been given of the isolation and chemical properties of *meso*-αβ-divinyldibenzyl. The present communication is primarily concerned with the presentation of some crystallographic data and a discussion of the molecular configuration of this new hydrocarbon together with that of the isomeric αβ-diethylidenedibenzyl of which only a bare mention exists in the literature (Stern, *Monatsh.*, 1905, **26**, 1565). The results are compared with those similarly derived for the two structural analogues *meso*-αβ-diethyldibenzyl and *trans*-αβ-diethylstilbene which have already been examined crystallographically by Carlisle and Crowfoot (*J.*, 1941, 6) and with regard to their isomorphous relationship by Wessely and Welleba (*Ber.*, 1941, **74**, 777, 785). Although no attempt has as yet been made at a detailed *X*-ray analysis of crystal structure in this series, yet the present study illustrates the application of preliminary *X*-ray and ultra-violet absorption data to the elucidation of complex organic structures, and consideration is also given in this paper to such diverse but probably interrelated phenomena as isomorphism and oestrogenic activity in relation to variations of spatial configuration.

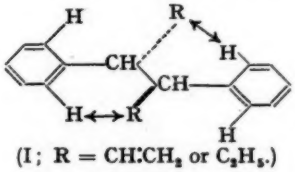
The hitherto unrecorded crystallographic properties of *meso*-αβ-divinyldibenzyl and αβ-diethylidenedibenzyl are given in the table, together with those obtained by Carlisle and Crowfoot (*loc. cit.*) for which the axes have been rearranged so as to refer all four crystal structures to the same space-group description $P2_1/n$.

Hydrocarbon.	Symbol.	M.p.	Unit cell dimensions (A.). *a.*	*b.*	*c.*	*β.*	No. of mols. in cell.	Space-group.	Molr. symmetry.	Oestrogen. activity, E.D.50.[3]
meso-αβ-Divinyldibenzyl ...	*A*	87°	6·71	18·76	5·85	110½°	2	$P2_1/n$	Centre	> 1 Mg. <10 mg.
meso-αβ-Diethyldibenzyl [1]...	*B*	89	7·00	19·27	5·75	110½	2	$P2_1/n$	Centre	< 1 Mg.
αβ-Diethylidenedibenzyl [2]...	*C*	101	6·47	18·70	6·16	110½	2	$P2_1/n$	Centre	> 1 Mg. <10 mg.
trans-αβ-Diethylstilbene [1] ...	*D*	71	12·38	20·18	7·66	131	4	$P2_1/n$	Centre or asymm.	~50 Mg.

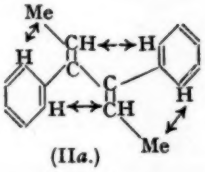
[1] Crystallographic data from Carlisle and Crowfoot (*loc. cit.*).
[2] Hydrocarbon of Stern (*loc. cit.*) and Lawson *et al.* (unpublished).
[3] Data obtained at the Courtauld Institute of Biochemistry, E.D.50 being defined as the dose necessary to effect 50% response in spayed rats after injection by a standard procedure (cf. this vol., p. 1116).

The crystalline modification of αβ-divinyldibenzyl of m. p. 87° must be assigned the *meso*-structure, since the crystal data require that each of the two molecules in the unit cell should possess a centre of symmetry. As might therefore be expected, the unit cell dimensions closely resemble those of *meso*-αβ-diethyldibenzyl, and it appears likely from the absence of any depression of mixed melting points that the two hydrocarbons form mixed crystals. Isomorphous replacement of vinyl by ethyl groups is evidently permitted in the *meso*-αβ-dialkyldibenzyl structure, and in fact the two molecules must possess almost identical shape. The presence of a centre of symmetry implies that the two benzene rings are parallel and that the three single bonds connecting the rings are all in one plane which may or may not contain the benzene rings. Coplanarity of these rings may, however, be excluded by inspection of Stuart models on account of the close approach of the substituent ethyl or vinyl groups to the *o*-hydrogen atoms of the benzene rings [cf. (I), where steric interference with a coplanar dibenzyl skeleton is indicated by double-headed arrows]. It seems certain therefore that both *meso*-αβ-divinyldibenzyl and *meso*-αβ-diethyldibenzyl exist in the " staggered " configuration characteristic of dibenzyl itself

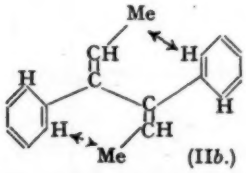
(Robertson, *Proc. Roy. Soc.*, 1935, *A*, **150**, 348; Jeffrey, *ibid.*, 1947, *A*, **188**, 222), in which the plane of the central bonds is approximately perpendicular to the planes of the benzene rings. In the crystal structure of dibenzyl this angle was measured accurately to be 70°, instead of the right angle which is to be expected in the isolated molecule. It has been suggested (Jeffrey, *loc. cit.*) that in the crystal the dibenzyl molecules can pack together with minimal lattice energy if the angle is 70° rather than 90°, and similar considerations may also be involved in the dialkyldibenzyl structures. The ethyl or vinyl substituents must be symmetrically disposed about the centre of the molecule at some orientation governed by the balance of steric requirements of atoms in the same and adjacent molecules.



(I; R = $CH{:}CH_2$ or C_2H_5.)



(II*a*.)



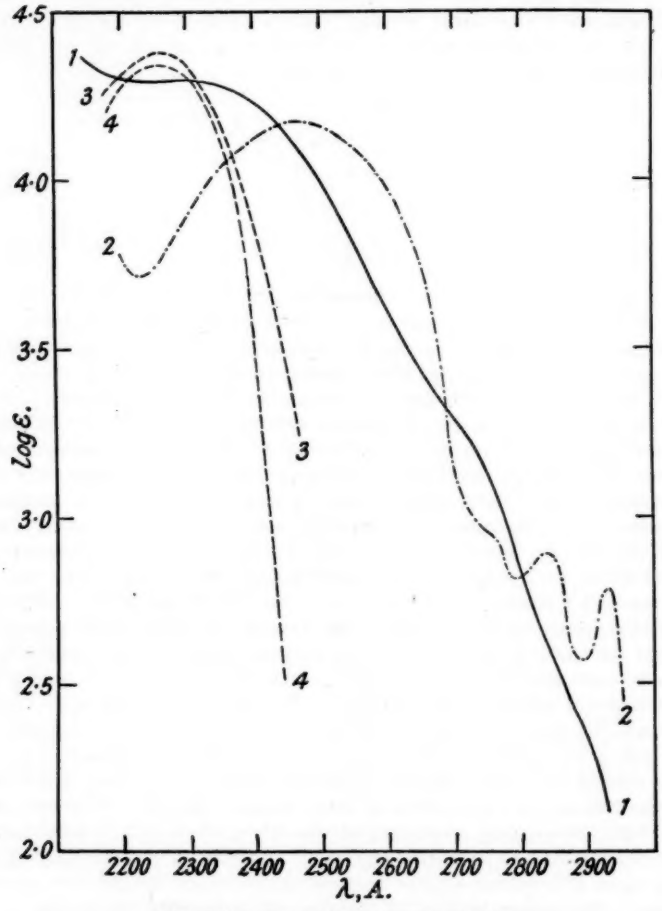
(II*b*.)

The spatial configuration of αβ-diethylidenedibenzyl of m. p. 101°, the conjugated double bond isomer of αβ-divinyldibenzyl, provides a more complicated problem. Although the crystallographic data reveal that the molecule possesses a centre of symmetry, two distinct forms both fulfilling this requirement are theoretically possible because of *cis-trans*-isomerism of the aliphatic hexadiene chain. These are the *trans-trans* form (II*a*) and the *cis-cis* form (II*b*).

The similarity in cell dimensions to the diethyl and the divinyl compound suggests that this molecule also has a staggered dibenzyl type of structure. On the other hand, the butadiene-type conjugation between the two double bonds, and the styrene-type conjugation of each double bond with a benzene ring, would induce the molecule to attain a completely coplanar configuration in order to gain the maximum resonance energy, in so far as such an arrangement is compatible with the van der Waals radii of atoms indicated by the double arrows in (II*a*) and (II*b*). That the structure cannot be entirely planar is immediately apparent from Stuart models (cf. also Zechmeister and LeRosen, *J. Amer. Chem. Soc.*, 1942, **64**, 2755). A further inference from the presence of a centre of symmetry in the molecule is that the two double bonds of the hexadiene chain and the three single bonds connecting the benzene rings must all lie in one plane, as also must the carbon atoms of the methyl groups if the ethylenic substituents are to retain their customary planar trigonal valency distribution. The aliphatic hexadiene chain is therefore completely planar, and the relief of the steric hindrance involved in a fully coplanar molecule must be brought about solely by rotation of the benzene rings out of the plane of the double bonds. The molecule thus retains the full butadiene-type resonance unimpaired, while the conjugation energy of each of the two styrene systems is to some degree reduced. This condition is reflected also in the ultra-violet absorption spectrum of the molecule which is recorded in the figure. The spectrum differs from that of a typical sterically unhindered styrene derivative but shows a main region of selective absorption similar to that observed for simple conjugated hexadienes. Considerable general absorption occurs at greater wave-lengths (including a shoulder near 2750 A.), and this is presumably associated with the reduced additional conjugation of the double bonds with the non-coplanar benzene rings. Similarly, the reported addition of *two* atoms of bromine to the doubly unsaturated compound (Stern, *loc. cit.*) may be cited as evidence for the presence of a typical aliphatic conjugated diene system in the molecule, while the coloration produced on mixing the hydrocarbon with *s*-trinitrobenzene in chloroform solution recalls the behaviour of substituted styrenes generally.

In arriving at a decision between the *trans-trans*- and the *cis-cis*-configurations for the molecule, the following considerations are involved. In the fully planar *trans-trans* model (II*a*) the steric hindrance affects all four *ortho*-positions and could be conveniently reduced by rotating the benzene rings about the single bonds to which they are attached. If one half of the molecule is rotated about the central single bond instead, steric interference with one of the two *ortho*-positions of each benzene ring would still occur, and the latter rotation is therefore regarded as an unlikely alternative. These conclusions are in accord with the observed symmetry properties of the molecule, which exclude the possibility of rotation about the central bond. On the other hand, it is found on examination of the *cis-cis* model that rotation of the benzene rings alone is hardly sufficient to remove all steric hindrance, for in the extreme perpendicular arrangement (III*b*) the carbon atoms of the methyl groups would still approach the benzene rings to a distance of only about 2·5 A. Moreover, such rotation would in any case

appear to be the more unlikely concession to steric requirements in the *cis-cis* structure, involving as it does the sacrifice of some resonance energy in the *two* fully planar styrene systems in favour of *one* butadiene conjugation kept intact. An unhindered and energetically stable molecule of the *cis-cis*-configuration could therefore only be obtained by rotation about the central single bond, resulting in asymmetry contrary to the experimental facts.



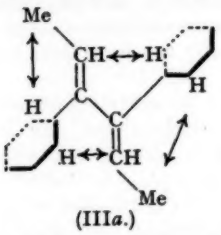
Absorption spectra in n-*hexane solution.*

1, 3 : 4-*Diphenylhexa*-2 : 4-*diene* (*αβ-diethylidenedibenzyl*).
2, 1-*Phenylpropylene.*
3, *Hexa*-2 : 4-*diene* (*in ethanol*; *after Booker, Evans, and Gillam,* J., 1940, 1453).
4, 2 : 3-*Dimethylbuta*-1 : 3-*diene* (*in methyl*cyclo*hexane*; *after Scheibe and Pummerer,* Ber., 1927, **60**, 2163).

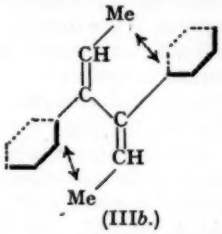
It appears reasonable therefore to conclude that the αβ-diethylidenedibenzyl of m. p. 101° has the *trans-trans*-configuration, which is presumably associated with greater stability than the hypothetical *cis-cis*-. In the *trans-trans* model (III*a*), rotation of the benzene rings by some 50° out of the plane of the double bonds would be sufficient to remove most of the steric interference (cf. Zechmeister and LeRosen, *loc. cit.*), giving rise to a centrosymmetric molecule which has the full resonance energy of a coplanar butadiene derivative and some appreciable fraction of the styrene conjugation. The shape of the dibenzyl skeleton may therefore be expected to be intermediate between the hypothetical coplanar and the staggered arrangements of the divinyl and diethyl compounds.

In contrast to diethyldibenzyl, diethylidenedibenzyl was observed to depress the m. p. of the divinyl derivative, although there are marked similarities in unit-cell dimensions. The lengths of the long *b*-axes in particular, which must be closely related geometrically to twice the length of the long axes of the molecules, are almost identical in the two isomers. It would seem

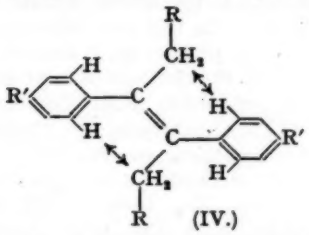
a tempting speculation to attribute the lengthening of the *c*-axis in the conjugated compound to the greater spatial extension of the fully coplanar six-carbon zigzag chain in its molecule, and



(III*a*.)



(III*b*.)



(IV.)

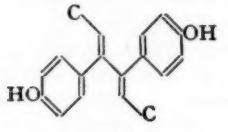
again to suspect a connection between the shortening of the *a*-axis and the general flattening of the structure which is demanded by resonance theory.

The molecular shape of *trans*-αβ-diethylstilbene (IV; R = Me, R′ = H) must finally be considered as the remaining structure of the series under discussion. Except for the two methyl carbons R, the whole molecule will again seek to attain coplanarity so as to gain the full resonance energy of stilbene-type conjugation, but some rotation of the benzene rings out of the plane of the ethylenic centre is required by the steric interferences indicated by double-headed arrows in the diagram. The reality of this steric hindrance (geometrically similar to those discussed above) and its inhibitory effect on electronic resonance have already been inferred from the observed ultra-violet absorption spectrum of the corresponding dimethylstilbene (IV; R = R′ = H) by Lewis and Calvin (*Chem. Reviews*, **1939**, **25**, 302) and by Jones (*J. Amer. Chem. Soc.*, **1943**, **65**, 1818). The benzene rings are presumably rotated symmetrically and kept parallel, by analogy with the centrosymmetrical character of the related *trans-pp′*-dihydroxy-αβ-diethylstilbene dipropionate structure (IV; R = Me, R′ = CH_2·CH_2·CO_2) (Carlisle and Crowfoot, *loc. cit.*). The existence or otherwise of a centre of symmetry in *trans*-αβ-diethylstilbene itself is not revealed by the available preliminary *X*-ray data alone. The space-group still requires the presence of four asymmetric units in the cell, but the hydrocarbon crystallises with four molecules in a doubled unit cell which differs from that of *meso*-αβ-diethyldibenzyl in much the same way in which stilbene differs from dibenzyl (cf. Prasad, Robertson, and Woodward, *Proc. Roy. Soc.*, **1935**, *A*, **154**, 187). The molecular shapes of *trans*-diethylstilbene and *meso*-diethyldibenzyl, both necessarily non-coplanar on steric grounds, correspond more closely, however, than those of stilbene and dibenzyl which are characterised by planar and staggered configurations, respectively. These spatial relationships are apparently illustrated by the properties of the mixed solid systems. Wessely and Welleba (*loc. cit.*) found that *trans*-αβ-dimethylstilbene (IV; R = R′ = H) and *meso*-αβ-dimethyldibenzyl (I; R = Me), and also *trans-pp′*-dimethoxy-αβ-diethylstilbene (IV; R = Me, R′ = OMe) and *meso-pp′*-dimethoxy-αβ-diethyldibenzyl gave rise to continuous series of mixed crystals, and analogous isomorphous behaviour may therefore be reasonably predicted for *trans*-αβ-diethylstilbene and *meso*-αβ-diethyldibenzyl as well. On the other hand, the planar stilbene and the non-planar dibenzyl molecules have recently been reported to be truly miscible only over a very small range in the crystalline state (Kofler and Brandstaetter, *Z. physikal. Chem.*, **1942**, *A*, **190**, 341).

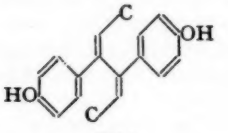
Unfortunately, no complete phase-diagrams could be obtained in the present work owing to the minute amounts of the diethyl- and diethylidene-dibenzyls at our disposal, and the non-availability of a specimen of diethylstilbene, but despite this shortcoming some interesting facts relating to the isomorphous relationships in the *meso*-αβ-divinyldibenzyl series emerge from the table. True isomorphism is obviously exhibited by the hydrocarbons *A* and *B*, a random mixture of which does not produce any depression of m. p. The compounds *A* and *C* possess extremely similar crystal geometry and physiological potency and are therefore probably isomorphous, too, in which case the depression of their mixed m. p. must be ascribed to the formation of a continuous mixed-crystal system of type III of the Bakhuis Roozeboom classification. On the other hand, *D* is crystallographically distinct from the other three members of the series but may nevertheless be presumed to give rise to an isomorphous phase-diagram in admixture with *B* in view of Wessely and Welleba's corresponding results on two pairs of homologous compounds (cf. above). Continuous mixed-crystal formation is evidently not a function of strictly analogous lattice geometry alone. It may well be that in the mixtures of *B* with *D* there is only a small energy gradient opposing the configurational adjustments required in the shape of either or both molecules for the formation of a mixed-crystal lattice, the resulting intra-molecular instability being more than compensated for by a gain in

lattice energy. Correns and others (*Chemie*, 1944, **57**, 29) have recently suggested on more general gounds that the idealised concept of isomorphism should be abandoned or qualified by reference to the several specific criteria such as miscibility, crystal geometry, and physiological activity. On this view, all four hydrocarbons of the table constitute an isomorphous series with respect to physiological activity and probably also miscibility, but only the first three appear to satisfy the criterion of analogous crystal structure.

In conclusion, it may be pointed out that the arguments relating to the spatial configuration of the hydrocarbons are equally applicable to their *pp'*-dihydroxy-derivatives, the highly active synthetic oestrogens. This circumstance is of some interest in connection with the marked dependence of high oestrogenic activity on stereochemical factors (cf. Dodds *et al.*, *Proc. Roy. Soc.*, 1944, *B*, **132**, 83; Shoppee, *Nature*, 1947, **160**, 64). In particular, it is now revealed that the true structure of dienoestrol, the centrosymmetrical *pp'*-dihydroxy-derivative of αβ-diethylidenedibenzyl (cf. Carlisle and Crowfoot, *loc. cit.*), must be *trans-trans* (V), and not the conventionally depicted *cis-cis* of the structure (VI) which has been designed to afford a superficial resemblance on paper to the space-arrangement of the nuclear carbon atoms in the natural oestrogenic hormone oestradiol (VII). Similarly, the conformation of the single bonds contained in the two ethyl groups of stilboestrol must be expected to approximate to a regular zigzag rather than to the S-shaped configuration of the " corresponding " atoms in the tetracyclic oestradiol structure, in order to offer the least possible steric opposition to a coplanar arrangement of the stilbene skeleton. Again, the principle of maximum resonance energy demands that the highly active *oo'*-αβ-tetramethylstilboestrol molecule (Bretschneider *et al.*, *Ber.*, 1941, **74**, 571; Hudson and Walton, *J.*, 1946, 85) should be near-planar with the ring configuration (VIII), offering no close analogy to the skeletal arrangement of the natural hormone. Scale models suggest, however, that the thickness of the highly active synthetic molecules is in every case similar to that of oestradiol itself, whereas some reported cases of feeble activity appear to differ in this respect (cf. Dodds, *loc. cit.*, and *ibid.*, 1939, *B*, **127**, 141).



(V.)



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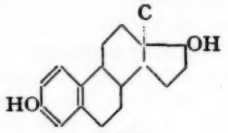
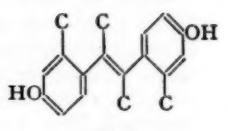


(VII.)



(VIII.)

Experimental.

meso-*αβ-Divinyldibenzyl*.—From petrol were obtained well-formed crystals of the monoclinic system, tabular on {010} with {101} and {001}. Careful indexing of single-crystal oscillation phtographs about the principal axes, in revealing the systematic extinctions characteristic of the space-group $P2_1/n$, showed that the two molecules in the unit cell were centrosymmetric.

meso-*αβ-Diethylidenedibenzyl* (trans-trans-3 : 4-*Diphenylhexa*-2 : 4-*diene*).—An almost pure sample of the hydrocarbon (small leaflets, m. p. 99—100°; cf. Stern, *loc. cit.*, who described shiny leaflets of m. p. 99°) was provided by Mr. W. Lawson of the Courtauld Institute of Biochemistry (Middlesex Hospital Medical School). After recrystallisation from ethanol, needles of m. p. 101—102° were obtained which depressed the m. p. of *meso-αβ*-divinyldibenzyl below 80°. The crystals, which were poorly formed, were tabular on {001} and elongated along [10$\bar{1}$]. As with the previous compound, the indexing of oscillation photographs identified the space-group as $P2_1/n$ with two centrosymmetric molecules in the unit cell. The ultra-violet absorption spectrum, except for the disappearance of a small shoulder near 2825 A., did not change after one further crystallisation. A yellow colour but no compound crystals were obtained on mixing with 2 mols. of *s*-trinitrobenzene in concentrated chloroform solution.

The authors would like to express their thanks to Professor E. C. Dodds, F.R.S., for communicating his data on oestrogenic activity, and to Dr. D. Crowfoot Hodgkin, F.R.S., and Mr. W. Lawson for gifts of diethyldibenzyl and diethylidenedibenzyl respectively. The work described in this paper arose out of a programme of fundamental research undertaken by the Board of the British Rubber Producers' Research Association.

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223. *Absorption Spectra of Dicinnamyl and Some Related Compounds.*

By H. P. Koch.

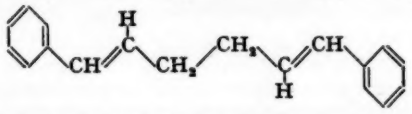
Comparative measurements are presented of the ultra-violet absorption spectra of both solutions and oriented crystals of 1 : 6-diphenylhexa-1 : 5-diene (dicinnamyl) and 1 : 10-diphenyldeca-1 : 9-diene, and of solutions of 1-propenylbenzene and 1 : 4-diphenylhexa-1 : 5-diene. All these hydrocarbons exhibit high-intensity absorption characteristic of *trans*-substituted styrenes. Two main wave-length regions of selective absorption may be recognised, one (2950—2750 A.) apparently associated with light vibrating in the plane of the benzene ring at right angles to the long axis of the styrene chromophore, and the other (2700—2300 A.) due to light vibrations in the same plane but normal to the above, *i.e.*, parallel to the direction of greatest extension of the styrene chain. The perpendicular 2950—2750 A. absorption exhibits characteristic fine-structure which is completely unaffected in the solution spectra by the second-order differences between the structural environments of the above hydrocarbon chromophores, although the band-heads are shifted to slightly longer wave-lengths in the crystalline state. The longitudinal 2700—2300 A. absorption region displays minor variations among the different compounds which must be attributed, at least in part, to weight or chain-length effects. Chromophore interaction across the three central single bonds of dicinnamyl is either non-existent or slight, precluding any kind of conjugation between the two styrene systems in the molecule.

The marked colour difference between lycopene and β-carotene is ascribed, not to incomplete electronic insulation of 1 : 5-spaced double bonds in lycopene, but to steric hindrance of conjugation in β-carotene.

X-Ray evidence recently presented by Jeffrey (*Proc. Roy. Soc.*, 1947, *A*, **188**, 222) has demonstrated the existence of abnormal bond-character associated with systems of two unsaturated groups separated by three single bonds. In both geranylamine hydrochloride and dibenzyl a significant shortening of the central single bonds was observed, and some degree of electronic interaction between the unsaturated groups was believed to accompany the unusual effect (cf. Bateman and Jeffrey, *Nature*, 1943, **152**, 446; Jeffrey, *Proc. Roy. Soc.*, 1945, *A*, **183**, 388). It was therefore to be expected that the peculiar bond structure would be reflected in the first ultra-violet absorption region of these compounds, which is generally considered highly characteristic of the energy levels of unsaturation electrons. No such correlation between abnormal bond length and absorption spectra could, however, be detected. Neither geranylamine hydrochloride (Bateman and Koch, *J.*, 1944, 600) nor dibenzyl (Ley and Dirking, *Ber.*, 1934, **67**, 1331; cf. Jones, *Chem. Reviews*, 1943, **32**, 1) exhibits quartz-ultra-violet absorption in any way suggestive of imperfect insulation between the unsaturated chromophores. Similarly negative evidence is obtained on comparing the absorption of αβ-divinyldibenzyl (a derivative of hexa-1 : 5-diene as well as of dibenzyl) with that of allylbenzene in the same spectral region (cf. this vol., p. 1116). These spectroscopic facts appear to be incompatible with the view that the bond abnormality is associated with a gain in resonance energy due to conjugation across three single bonds. Since the first *intense* ($N \longrightarrow V$) absorption bands of all the above compounds have their actual peaks located in the region of 2000 A. or beyond where precise quantitative measurement becomes difficult, it was felt desirable to confirm this result with systems giving rise to well-defined maxima of intense absorption within the range of the usual spectrophotometric equipment. Accordingly, the ultra-violet survey has now been extended to dicinnamyl and similar molecules, and the observed electronic spectra have been considered in some detail.

Dicinnamyl (I) is structurally derived from dibenzyl by substitution of styryl groups for the two benzene rings, and the greater degree of conjugation of the styrene system over benzene causes the first intense absorption band of dicinnamyl to occur at considerably longer wave-lengths in the quartz-ultra-violet region. In order to ascertain the presence or otherwise of chromophore interaction in the molecule, it is necessary to relate the spectrum of dicinnamyl to that of other β-substituted styrenes of different intramolecular environment, with due attention to the possible influence of steric factors. Complexities of geometrical isomerism hardly exist, since the styrene system differs from the analogous butadiene chromophore in the important respect that it does not admit of spectrally significant *cis-trans* isomerism about the single bond linking the two conjugated unsaturated centres (cf. Sugden and Walsh, *Trans. Faraday Soc.*, 1945, **41**, 76) : only one form of styrene is possible. Again, only one stable modification of dicinnamyl (m. p. 81°), usually prepared from the crystalline *trans*-cinnamyl halides, is known (cf. this vol., p. 1112), and this may undoubtedly be assigned the *trans-trans*-configuration (I) with regard to the double bonds. An extended zigzag chain formulation is in accord with the

centrosymmetrical character and unit-cell dimensions of the molecule in the crystalline state, and the solution spectra plotted on a logarithmic scale in Fig. 1 show that the two styrene

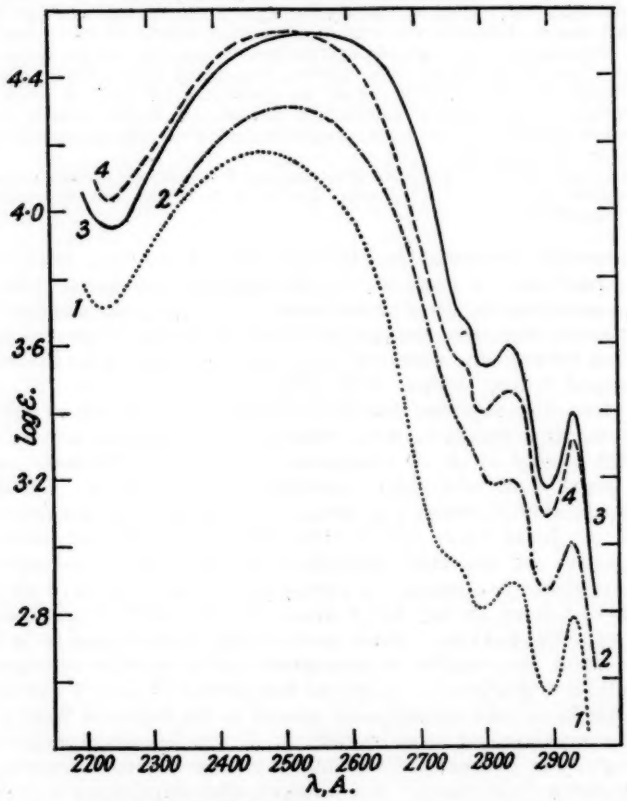


(I.)

chromophores in dicinnamyl together absorb rather more than twice as strongly as one styrene system in propenylbenzene or styrene itself (for the latter, cf. Jones, *loc. cit.*). These spectroscopic relationships are incompatible with a *cis*-structure of dicinnamyl in which the

FIG. 1.

Absorption spectra in n-*hexane solution.*



1, 1-*Propenylbenzene.*
2, 1 : 4-*Diphenylhexa*-1 : 5-*diene.*
3, 1 : 6-*Diphenylhexa*-1 : 5-*diene* (*dicinnamyl*).
4, 1 : 10-*Diphenyldeca*-1 : 9-*diene.*

styrene-type conjugation would be considerably weakened by the steric interference between an aromatic *ortho*-carbon and a methylene group in the coplanar form required for full electronic resonance. According to the relevant scale models already examined in the preceding paper and also by Zechmeister and LeRosen (*J. Amer. Chem. Soc.*, 1942, **64**, 2755), in similar contexts, a *cis*-substituted styrene demands appreciable rotation of the benzene ring out of the molecular plane for the removal of such interference between the van der Waals radii of non-bonded groups; and reference to such spectra as those of stilbene and αβ-dimethylstilbene (Jones, *ibid.*, 1943, **65**, 1818) provides a good illustration of the well-known fact that steric incompatibility of the geometrical type under discussion always produces a marked reduction in light-absorption properties.

The same arguments may be applied to the ultra-violet spectrum of the homologous hydrocarbon 1 : 10-diphenyldeca-1 : 9-diene (II) of m. p. 53°, which is also shown in Fig. 1.

This absorption strongly resembles that of dicinnamyl, and the *trans-trans*-structure of the higher homologue cannot therefore be in doubt. By analogy, the two liquid hydrocarbons 1-propenylbenzene (III) and 1 : 4-diphenylhexa-1 : 5-diene (*iso*dicinnamyl; IV) of Fig. 1 are likewise regarded as the *trans*-isomers.

Although the light-absorption characteristics of styrene itself are well known, 1-propenylbenzene (III) is the only example of the substituted styrenes illustrated in Fig. 1 for which the spectrum has been determined previously (cf. Jones, *loc. cit.*). This hydrocarbon was obtained by alkaline isomerisation of allylbenzene. 1 : 10-Diphenyldeca-1 : 9-diene (II) was prepared by standard methods, and the preparation of spectroscopically pure (non-fluorescent) samples of dicinnamyl (I) and *iso*dicinnamyl (IV) has been described in a preceding paper (this vol., p. 1111). It should be mentioned that Smakula (*Angew. Chem.*, 1934, **47**, 657) has reported maximum extinction data for 3 : 4-dihydroxy-1 : 6-diphenylhexa-1 : 5-diene (V) which suggest a close resemblance between the ultra-violet absorption of this dihydroxydicinnamyl ($\lambda_{max.} = 2560$ A., $\varepsilon = 28{,}000$ in ethanol) and that of dicinnamyl itself.

$CHPh{:}CH{\cdot}[CH_2]_6{\cdot}CH{:}CHPh$ (II.) $CHPh{:}CH{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH{:}CHPh$ (V.)

$CHPh{:}CH{\cdot}CH_3$ (III.) $CHPh{:}CH{\cdot}CH(OH){\cdot}CH_3$ (VI.)

$CHPh{:}CH{\cdot}CH_2{\cdot}CHPh{\cdot}CH{:}CH_2$ (IV.)

All the spectra reproduced in Fig. 1 exhibit two main regions of selective absorption, the first between 2950 and 2750 A. with well-defined vibrational fine-structure peaks at 2930 and 2840 A., and the second between 2700 and 2300 A. with a rather broad band-head more than ten times as intense as the long-wave region. Such light-absorption properties are apparently characteristic of all β-alkyl-substituted styrenes (cf. Ramart-Lucas and Amagat, *Bull. Soc. chim.*, 1932, **51**, 965; Braude, Jones, and Stern, *J.*, 1946, 396) and also similar to those of benzaldehyde (Walsh, *Trans. Faraday Soc.*, 1946, **42**, 62), and their origin may be explained as follows: The 2700—2300 A. region is almost certainly analogous to the intense $N \longrightarrow V$ transition of butadiene around 2200 A. and therefore to be associated with resonance of the type (A), the absorption being polarised in the plane of the styrene chromophore with the light vibrations in a direction parallel to its long axis. The 2950—2750 A. region, on the other hand, with a

(and two *o*-quinonoid structures) (A.)

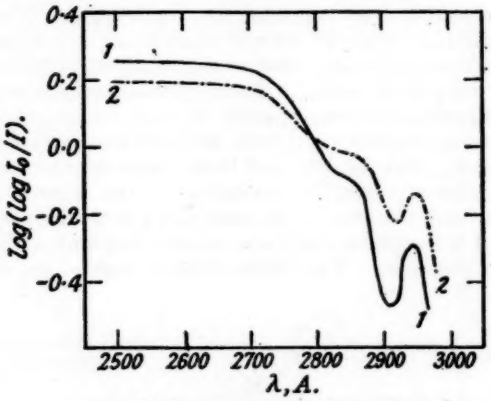
fine-structure separation of 1010 cm.$^{-1}$ corresponding to the symmetrical " breathing " frequency of the benzene ring, is analogous to the " forbidden " benzene absorption near 2600 A., facilitated in the derivatives by the decreased symmetry resulting from substitution in the aromatic ring. This absorption is presumably polarised in the same plane but with the electric vector normal to the above, so that the two absorption systems correspond to the mutually perpendicular " *x* " and " *y* " bands believed to be associated with all complex aromatic hydrocarbons and similar chromophores (Lewis and Calvin, *Chem. Reviews*, 1939, **25**, 273; Jones, *ibid.*, 1947, **41**, 353).

Interesting confirmation of the above directional assignment of absorption bands is obtained on measuring the ultra-violet transmission of a thin oriented layer of dicinnamyl or 1 : 10-diphenyldeca-1 : 9-diene in the crystalline state. Dicinnamyl generally crystallises in thin flakes; and preliminary *X*-ray crystallographic examination by Mr. S. C. Nyburg, together with trial packing experiments and observations on diffuse thermal reflections kindly carried out by Dr. K. Lonsdale, have shown that the long axis of the molecules lies almost at right angles to the face of the flakes. When molten and squeezed between plates of glass or quartz to a thickness of the order of 1 μ, the resultant film of dicinnamyl gives rise on freezing to a number of crystalline regions accounting for at least 90% of the bulk according to *X*-ray photographs, each region being essentially a single flake-like crystal with its face roughly parallel to the containing surface. To a first approximation, therefore, the hydrocarbon may be regarded as oriented with the length of the molecule and also the long axis of the styrene chromophores roughly normal to the flat surface of the resulting sandwich structure. Similar orientation was observed by Scheibe, Hartwig, and Mueller (*Z. Elektrochem.*, 1943, **49**, 372; cf. also Krishnan and Seshan, *Proc. Indian Acad. Sci.*, 1938, *A*, **8**, 487), who found that hexamethylbenzene crystallised between quartz plates with the aromatic ring planes of the molecules perpendicular to the plane of the optical flats. On passing a beam of unpolarised ultra-violet light across the dicinnamyl film at a right angle, all the electric vectors, being directed at right angles to the

direction of propagation of the light, will then be polarised in the plane of the flat face, *i.e.*, more or less *normal* to the orientation of the long axis of the styrene chromophore. Consequently, selective absorption in the 2700—2300 A. region associated with the resonance (A) depicted above and requiring vector polarisation *parallel* to this axis should be greatly weakened or suppressed. Analogous optical phenomena were encountered by Krishnan and Seshan (*loc. cit.*) in their experiments on crystals of chrysene which did not absorb ultra-violet light vibrating at right angles to the molecular planes.

The observed spectrum of the dicinnamyl " sandwich " is plotted as curve (1) of Fig. 2 and may be compared with the corresponding solution spectrum, although absolute extinction coefficients could not be determined owing to the uncertain losses incurred through scattering and reflection. The characteristic first absorption region at 2950—2750 A. is still clearly discernible, but the high-intensity maximum between 2700 and 2300 A. has disappeared and been replaced by considerably reduced and apparently continuous extinction which may be attributed in part to both anomalous and Rayleigh scattering in the crystalline film, the latter being slightly opaque to visible light. Relative to the long-wave bands as an internal standard, the short-wave absorption attributed to excitation associated with the resonance (A) is evidently much weaker in the oriented crystal flat than in solution, in accordance with expectation. The

FIG. 2.



1, ——— 1 : 6-*Diphenylhexa*-1 : 5-*diene* (*dicinnamyl*).
2, —·— 1 : 10-*Diphenyldeca*-1 : 9-*diene*.

2950—2750 A. absorption of the film is quite strong and therefore presumably polarised in perpendicular directions where electric vectors are available for the requisite excitation *normal* to the long axis of the styrene chromophore. At the same time, the vibrational fine-structure peaks are shifted from 2930 and 2840 A. in solution to 2945 and 2860 A. in the crystal, their characteristic separation being maintained at 1010 wave-numbers, the " breathing" frequency of the benzene ring. Like shifts of 15—20 A. take place between the ultra-violet absorption bands of hexamethylbenzene as measured in solution and in the crystalline state (Scheibe *et al.*, *loc. cit.*).

Curve (2) of Fig. 2 represents the transmission of a crystalline film of 1 : 10-diphenyldeca-1 : 9-diene (III), set up analogously to the above. The spectrum is extremely similar to that of dicinnamyl and it may be presumed that here, too, the long axes of the styrene chromophores are oriented more or less at right angles to the characteristic flat face of the crystals which is doubtless developed in a direction parallel to the quartz plates.

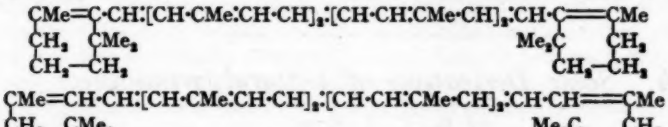
On careful comparison of the solution spectra of the four β-substituted styrenes plotted in Fig. 1, the location of the perpendicular benzenoid maxima was found to be remarkably constant at 2930 and 2840 A. On the other hand, significant second-order variations do occur between the centres of the longitudinal styrene absorptions in the short-wave region. At first sight it seemed, in fact, that intramolecular electronic interaction in the 1 : 5-unsaturated systems was indicated by the gradual shift from 2470 to 2550 A. and simultaneous intensification of the extinction calculated per styrene group in the series propenylbenzene <*iso*dicinnamyl <dicinnamyl. These small shifts appeared to be the more significant in view of the work of Ramart-Lucas and Amagat (*loc. cit.*), who compared styrene and β-octylstyrene and claimed that the length of chain attached had no influence on the ultra-violet absorption

properties. Subsequent examination of the 1 : 9-unsaturated diphenyldecadiene, however, strongly supported the view that the observed bathochromic effects must be due, in part at least, to other causes. The absorption of this higher homologue [curve (4) of Fig. 1] is so similar to the dicinnamyl spectrum, particularly as regards the short-wave slope of the main band and its peak intensity, that the remaining relative shift between the respective absorption centres (located at 2505 and 2550 A.) may scarcely be considered sufficient spectroscopic evidence for electronic interaction of the two styrene systems in the dicinnamyl molecule. If anything, the spectra suggest that there is a definite small weight or chain-length effect which causes the styrene chromophores in diphenyldecadiene to absorb relatively more strongly and at longer waves than they do in propenylbenzene; and it must remain an open question in how far the displacements of the *iso*dicinnamyl and dicinnamyl absorptions from the propenylbenzene position are due to similar weight effects or to slight interaction of the unsaturation electrons across the three central single bonds as envisaged by Bateman and Jeffrey (*loc. cit.*).

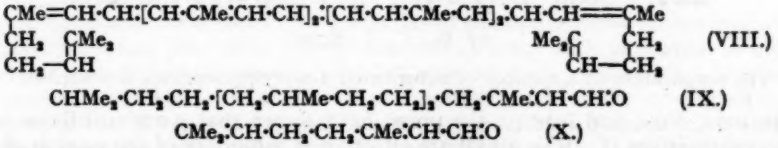
Walsh (*Proc. Roy. Soc.*, 1947, *A*, **191**, 32) has examined the absorption of styrene in the vapour phase and found that the broad short-wave maximum was split into two distinct humps with a separation of 1650 cm.$^{-1}$ corresponding to the carbon–carbon double-bond stretching frequency (cf. Hausser, *Z. tech. Physik*, 1934, **15**, 10). There can be little doubt that all the strong continua between 2700 and 2300 A. shown in the spectral curves of Fig. 1 arise from the superposition of discrete vibrational sub-levels, and Braude, Jones, and Stern (*loc. cit.*) have in fact resolved the solution spectrum of styrylmethylcarbinol (VI) into two band-heads at 2510 and 2480 A., although the dicinnamyl maximum could not be resolved in the present work with the aid of a Hilger medium spectrograph. The rather unsymmetrical broadening and displacement of the styrene-type spectra of *iso*dicinnamyl, dicinnamyl, and diphenyldecadiene may therefore conceivably be due to changes in the energy or relative importance of their separate vibrational levels rather than to a genuine shift of electronic activation. If indeed the three central bonds of dicinnamyl are shortened for whatever cause, the unusual valency orbital hybridisation involved might be expected to have some effect also on the vibrational characteristics of the adjacent double bonds, in both ground and excited states. So-called chain-length effects may well be similarly correlated with weighting effects on the valence vibration determining the contour of the electronic bands.

Attention should also be drawn to the fact that the styrene-analogous butadiene absorption region around 2200 A. is equally subject to second-order chain-length effects. Thus, the well-known conjugated linoleic compounds which may be regarded as terminally long-chain di-substituted butadienes all absorb maximally near 2320 A., whereas simple hexa-2 : 4-dienes are characterised by more short-wave absorption peaks in the region of 2275 A. (cf. Bolland and Koch, *J.*, 1945, 445). These shifts are small but nevertheless significant.

To conclude this spectral survey of 1 : 5-spaced hydrocarbon chromophores, an apparent exception to the general failure to detect appreciable interaction effects remains to be discussed. A well-known and striking colour change (band-shifts from 4835 and 4520 A. to 5060 and 4740 A.) accompanies the structural change from β-carotene (VII) to lycopene (VIII); and since the two isomeric pigments are based on the same system of eleven double bonds in conjugation, the deeper colour of lycopene might be thought to be due to electronic interaction across three single bonds with the additional terminal double bonds of this molecule. The absorption bands of γ-carotene, which has one of the two end groups cyclised and the other terminally unsaturated, lie intermediate between those of (VII) and (VIII). A recent comparison of the ultra-violet

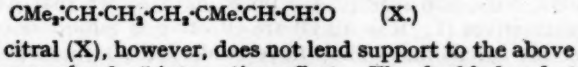


(VII.)



(VIII.)

(IX.)



(X.)

spectra of phytal (IX) and citral (X), however, does not lend support to the above interpretation of the spectral shifts in terms of a 1 : 5-interaction effect. The double-bond structure of the two open-chain αβ-unsaturated aldehydes (IX) and (X) differs exactly as (VII) differs from (VIII) by the absence or presence respectively of a terminal double bond in a position 1 : 5 to a conjugated chromophore, and yet both citral and phytal display maximum absorption at precisely

the same wave-length of 2380 A. (Burawoy, *J.*, 1941, 23; and unpublished work). The colour change between lycopene and β-carotene must evidently find its true explanation in terms of the cyclisation, as may be readily understood by reference to steric scale models. Although resonance stabilisation undoubtedly induces a fully coplanar zigzag arrangement of the entire system of eleven conjugated double bonds in lycopene (VIII), yet the scale model of β-carotene (VII) does not admit of coplanarity with respect to the two terminal double bonds forming part of the cyclic portion of the molecule on account of steric interference between the planar central chain and the methyl groups on the rings. The effective electronic conjugation of the cyclic double bonds of β-carotene is therefore reduced and the light absorption shifted to shorter wave-lengths relative to the lycopene pigment. Similar spectral abnormalities have been recorded for β-ionone, β-*cyclo*citral, and their crystalline semicarbazones (cf. Koch, *Chem. and Ind.*, 1942, **61**, 273); and the relatively small spectral shift associated with displacement out of conjugation of the two cyclic double bonds (from 4835 and 4520 A. in β-carotene to 4780 and 4475 A. in α-carotene) may be cited as further evidence of the small conjugating power of these double bonds due to inhibition by unfavourable steric conditions.

Experimental.

All spectra were recorded with the aid of a Spekker photometer attached to a Hilger small quartz spectrograph. Through the courtesy of Dr. D. M. Simpson, a Hilger medium spectrograph was also made available at the University of Cambridge for the examination of dicinnamyl, without, however, achieving any resolution of the broad absorption maximum. A detailed description of the *X*-ray work on dicinnamyl will be given elsewhere, but preliminary data are contained in a preceding communication (this vol., p. 1111).

1-*Propenylbenzene*.—Allylbenzene (5 g.; cf. this vol., p. 1116) was heated with potassium hydroxide (10 g.) in ethylene glycol solution (100 g.) for 1 hour at 160° in an atmosphere of nitrogen. After the addition of water and ether, the isomerised hydrocarbon was isolated from its ethereal solution in the usual manner and distilled at 171°/763 mm.; $n_D^{13.5°}$ 1·5510. 1-Propenylbenzene differs from allylbenzene in giving a yellow colour with *s*-trinitrobenzene in chloroform solution.

1 : 10-*Diphenyldeca*-1 : 9-*diene* (cf. Borsche and Wollemann, *Ber.*, 1911, **44**, 3185; Schmidt and Grosser, *ibid.*, 1942, **75**, 826).—Sebacophenone (48 g., prepared by Friedel–Crafts reaction between sebacyl chloride and benzene; m. p. 92·5—93·5°) was reduced in a boiling molar solution (300 c.c.) of aluminium *iso*propyl alcohol. After completion of the reaction (no more acetone distilling over) the bulk of the solvent was taken off at reduced pressure and the residue hydrolysed by addition of dilute hydrochloric acid. The resulting oil gradually solidified, and the colourless solid was crystallised from benzene after being washed with dilute acid and water. The purified 1 : 10-dihydroxy-1 : 10-diphenyldecane was only obtained as an amorphous powder, m. p. 63·5—69° (lit., 70—72°; needles, m. p. 72°), insoluble in petrol and extremely soluble in ethanol (Found: C, 80·9; H, 9·3. Calc. for $C_{22}H_{30}O_2$: C, 80·95; H, 9·25%) (this alcohol would hardly be expected to be homogeneous but rather a mixture of equal proportions of the DL- and the *meso*-form). Facile dehydration took place over potassium hydrogen sulphate at 160—180° under reduced pressure, and the resulting 1 : 10-diphenyldeca-1 : 9-diene began to crystallise soon after distillation at 150°/10^{-3} mm. The hydrocarbon was obtained in thin irregular plates from ethanol, m. p. 53·5° (lit., needles, 53°). It gave a yellow colour but no compound crystals on mixing with 2 mols. of *s*-trinitrobenzene in concentrated chloroform solution.

The author is indebted to Dr. A. D. Walsh for communicating his manuscript on styrene vapour spectra in advance of publication. Thanks are also due to Mr. S. C. Nyburg for the *X*-ray crystallographic measurements on dicinnamyl referred to in this investigation, which forms part of a programme of fundamental research undertaken by the Board of the British Rubber Producers' Research Association.

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48 Tewin Road, Welwyn Garden City, Herts. [*Received, July 31st*, 1947.]

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224. *Some Derivatives of 4-Styrylpyrimidine.*

By W. C. J. Ross.

The preparation of a number of substituted 4-styrylpyrimidines is described.

Haddow, Harris, Kon, and Roe (in the press) have shown that *p*-aminostilbene and certain of its *N*-alkyl derivatives (I; R = alkyl) are effective as inhibitors of the growth of the transplanted Walker rat carcinoma. They have studied the structural requirements for activity and find that the following items are essential: (1) a basic substituent (an amino- or alkylamino-group) in the *o*- or *p*-position in one ring; (2) an unsubstituted *p*-position in the other ring; (3) an unsubstituted ethylenic bridge; (4) a *trans*-stilbene structure. So far these requirements have been established only for a series of compounds in which both rings A and B are benzenoid.

It has now been considered desirable to extend the series to include compounds in which either ring A or ring B—or possibly both rings—are replaced by heterocyclic ring systems. In the present paper a series of compounds is described in which rings A and B have been separately replaced by a substituted pyrimidine nucleus.

Two methods have been described in the literature for the preparation of 4-styrylpyrimidine derivatives. Gabriel and Colman (*Ber.*, 1903, **36**, 3379) condensed 4-methylpyrimidine with benzaldehyde by heating them with zinc chloride at 150°. They also prepared 2 : 4-distyrylpyrimidine in a similar manner from 2 : 4-dimethylpyrimidine. Bergman and Johnson (*Ber.*, 1933, **66**, 1492) found that ethyl 2-hydroxy-4-methylpyrimidine-5-carboxylate condensed with benzaldehyde at 180° without a catalyst. Folkers, Harwood, and Johnson (*J. Amer. Chem. Soc.*, 1932, **54**, 3751) obtained 2-hydroxy-4-styryl-6-methyl-5 : 6-dihydropyrimidine-5-carboxylic ester by the acid-catalysed condensation of urea, ethyl acetoacetate, and cinnamaldehyde.

4-Methyluracil is recovered unchanged after being heated for two hours with benzaldehyde at 150° in the presence of zinc chloride. The same result is obtained when the methyluracil and benzaldehyde are heated under reflux with acetic anhydride, in alcoholic sodium hydroxide solution, or with piperidine. However, when they are heated with zinc chloride at 170° for three hours a reaction takes place, but the *product* is not the required 4-styryluracil for it has apparently been formed by the condensation of two molecules of methyluracil with one molecule of benzaldehyde. Only two of its three oxygen atoms are present as hydroxyl groups which can be replaced by chlorine atoms by treatment with phosphorus oxychloride. The *dichloro*-derivative reacts with piperidine to give a *dipiperidino*-compound. The parent dihydroxy-compound is insoluble in camphor, but the molecular weights of the dichloro- and the dipiperidino-compound confirm the above course of reaction. It is suggested that the parent compound may be provisionally assigned the xanthine-like structure (II; R = H).

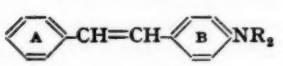
It has not proved possible to condense benzaldehyde with 4-methyluracil under conditions mild enough to avoid side reactions such as that already described. It is evident that the 4-methyl group in methyluracil is considerably less active than in the pyrimidine from which it is derived. In view of this an attempt was made to condense 5-nitro-4-methyluracil with benzaldehyde. As was expected, the presence of the nitro-group had the effect of activating the methyl group, and *5-nitro-4-styryluracil* (III; R = H) was readily obtained by using piperidine as catalyst. When excess of piperidine is used the styryl compound is isolated as its water-soluble piperidine salt. 5-Nitro-4-styryluracil is easily obtained from this salt by treatment with acids; it can also be obtained directly by condensation in alcoholic solution in the presence of a trace of piperidine, though under these conditions the reaction is much slower.

When an attempt is made to replace the hydroxyl groups in 5-nitro-4-styryluracil by chlorine atoms by heating with phosphorus oxychloride in the presence of dimethylaniline (Baddiley and Topham, *J.*, 1944, 678), a crystalline *compound*, $C_{16}H_{13}O_2N_4Cl$, is obtained. This is no doubt formed by the reaction of the active 6-chlorine atom which is first introduced with the dimethylaniline, and is formulated as (IV); it has been characterised by the preparation of its *2-piperidino*-derivative. It was at first thought that the dimethylaniline might have contained sufficient methylaniline to account for the direct formation of (IV), that is, without the need to assume prior elimination of a methyl group, but even after careful purification of the dimethylaniline the reaction still followed the same course. A similar reaction is described by Kawai and Miyoshi (*Sci. Papers. Inst. Phys. Chem. Res. Tokyo*, 1931, **16**, 20), who found that 2 : 4 : 6-trichloropyrimidine readily reacted with dimethylaniline to give 2 : 4 : 6-trimethylanilinopyrimidine.* The required dichloronitro-compound cannot be obtained by the action of phosphorus oxychloride in pyridine or by the use of thionyl chloride. 2 : 6-*Dichloro-5-nitro-4-styrylpyrimidine* was eventually obtained in good yield by heating 5-nitro-4-styryluracil with phosphorus oxychloride at 170° for two or three days.

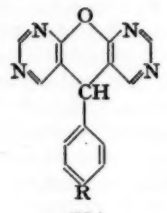
There is a considerable difference in the reactivity of the two chlorine atoms in 2 : 6-dichloro-5-nitro-4-styrylpyrimidine, for, while both atoms readily react with piperidine to give *5-nitro-2 : 6-dipiperidino-4-styrylpyrimidine*, only one chlorine atom is replaced by treatment with methanolic ammonia either in the cold or at the boiling point. It is shown below that the 6-chlorine atom is the more reactive, and thus the mono-amino-compound is *2-chloro-5-nitro-6-amino-4-styrylpyrimidine* (V). The mono-amine readily reacts with piperidine to give *5-nitro-2-piperidino-6-amino-4-styrylpyrimidine*. Reduction of the amine (V) with stannous chloride affords *2-chloro-5 : 6-diamino-4-styrylpyrimidine* which is a strong base. The strong

* *Added in Proof.*—More recently King, King, and Spensley (*J.*, 1947, 1247) have shown that a methylanilino-compound is formed when barbituric acid is chlorinated by Baddiley and Topham's method.

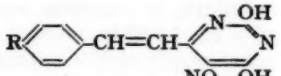
basicity of this diamine supports its formulation as a 5 : 6-diamine (cf. Gabriel and Colman, *Ber.*, 1901, **34**, 1234), and this is confirmed by the formation of *2-chloro-8-hydroxy-4-styrylpurine* when the base is heated with urea. The diamine also reacts with oxalic acid at 170°; the chlorine atom is replaced by a hydroxyl group and a lumazine type of compound is formed. The product, *2 : 8 : 9-trihydroxy-6-styrylpteridine* (VI), can be regarded as a leucopterin derivative. When the diamine is heated with methanolic ammonia for five hours at 200—210° no reaction occurs.



(I; R = H or alkyl.)



(II.)



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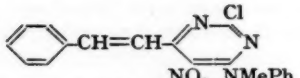

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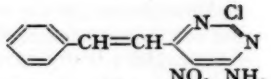
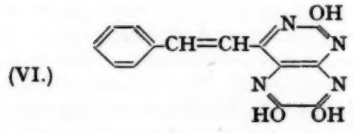
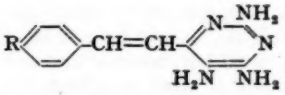


(V.)



(VI.)



(VII.)

If 2-chloro-5-nitro-6-amino-4-styrylpyrimidine (V) is heated with methanolic ammonia for seven hours at 220°, the remaining chlorine atom is replaced by an amino-group, but hydrolytic fission of the double bond occurs and 5-nitro-2 : 6-diamino-4-methylpyrimidine is formed. Under milder conditions—two hours at 110°—no fission occurs, and *5-nitro-2 : 6-diamino-4-styrylpyrimidine* is obtained. This compound can be reduced by stannous chloride in fuming hydrochloric acid to *2 : 5 : 6-triamino-4-styrylpyrimidine* (VII, R = H). This triamine is also a strong base, and when a solution of its stannichloride is treated with excess of ammonia a *monohydrochloride* can be isolated from the filtrate. In order to obtain the free base from this salt treatment with sodium hydroxide is necessary. The triamine has an interesting effect on the kidney of the rat (personal communication from Professor Haddow). It appears to induce an increase in kidney size similar to that produced by xanthopterin—a preliminary reference to the action of the pterin has been made by Haddow (*British Medical Bulletin*, 1947, **4**, 337).

2-Amino-8-hydroxy-4-styrylpurine is obtained when the triaminostyrylpyrimidine is heated with urea; it is soluble in dilute hydrochloric acid, from which it is precipitated as a monohydrate by addition of sodium acetate. The triamine also reacts with oxalic acid to give a lumazine derivative which can be regarded as *4-styryl-4-deoxyleucopterin*. The method of preparing this pterin derivative is similar to that used by Purrman in his synthesis of leucopterin from 2 : 4 : 5-triaminopyrimidine (*Annalen*, 1940, **544**, 182). The styryltriamine also reacts with barium glyoxylate in 70% sulphuric acid to give a compound with properties similar to those of xanthopterin (cf. Koschara's preparation of the pterin, *Z. physiol. Chem.*, 1943, **277**, 159), and with mesoxalic ester in acid media to give an acidic product (cf. Purrman's preparation of xanthopterincarboxylic acid, *Annalen*, 1941, **548**, 284).

p-Nitrobenzaldehyde also condenses with 4-methyluracil in the presence of zinc chloride to give a *product* which is regarded as having a xanthine-like structure (II; R = NO_2); it has been characterised by the preparation of the *dichloro-* and the *dipiperidino*-derivative. As in the previous instance difficulty was experienced in removing zinc salts from the products, and the analytical figures are not altogether satisfactory.

In contrast to the ease with which benzaldehyde condenses with 5-nitro-4-methyluracil in the presence of piperidine, *p*-nitrobenzaldehyde fails to condense under these conditions, and the reaction is not catalysed by mineral acids. *5-Nitro-4-p-nitrostyryluracil* (III; R = NO_2) is, however, readily obtained when the aldehyde, nitromethyluracil, and zinc chloride are heated at 150°. The dinitro-compound gives a water-soluble *piperidine* salt. When the uracil derivative (III; R = NO_2) is heated for several days at 170° with phosphorus oxychloride, *2 : 6-dichloro-5-nitro-4-p-nitrostyrylpyrimidine* is obtained. The introduction of the nitro-group into the *p*-position in the benzene ring has an interesting effect on the reactivity of the chlorine atom in the 6-position in the pyrimidine ring, for, while this chlorine atom in 2 : 6-dichloro-5-nitro-4-styrylpyrimidine readily reacts with ammonia in cold methanolic solution, the corresponding

chlorine atom in the *p*-nitro-derivative does not react even at boiling point. Both chlorine atoms react instantly with piperidine to give 5-*nitro*-2 : 6-*dipiperidino*-4-p-*nitrostyrylpyrimidine*. When the dichloro-compound is heated with methanolic ammonia at 110°, both chlorine atoms are replaced with formation of 5-*nitro*-2 : 6-*diamino*-4-p-*nitrostyrylpyrimidine*. Reduction of the dinitrodiamine with stannous chloride affords 2 : 5 : 6-*triamino*-4-p-*aminostyrylpyrimidine* (VII; R = NH_2).

When the tetra-amine is heated with oxalic acid at 140° a product which gives an insoluble sodium salt is obtained. This is probably an oxanilide type of derivative of the styrylleucopterin. When the amine (VII; R = NH_2) is heated with urea, an alkali-soluble product is obtained. The purification of the styryl- and aminostyryl-derivative of purines and pterins is very difficult owing to the tenacious retention of inorganic salts when they are precipitated from aqueous solutions: they are almost insoluble in the usual organic solvents.

p-Dimethylaminobenzaldehyde readily condenses with 5-nitro-4-methyluracil, giving the deep crimson 5-*nitro*-4-p-*dimethylaminostyryluracil* (III; R = NMe_2) which gives a brick-red *piperidine* salt. It has not so far proved possible to obtain the corresponding 2 : 6-dichloro-compound, possibly owing to a side reaction in which the chloro-compound first formed reacts with dimethylamino-group of another molecule as described above.

Experimental.

(All m. p.s were determined in sealed capillaries and are uncorrected.)

Condensation of Benzaldehyde with 4-Methyluracil.—4-Methyluracil, benzaldehyde, and finely powdered anhydrous zinc chloride (10 g. of each) were intimately mixed and gradually heated in an oil-bath, with occasional stirring, to 170°. The methyluracil quickly dissolved in the benzaldehyde, and after 3 hours at 170° all the zinc chloride had passed into solution and a clear brown liquid had resulted. After cooling, the material was dissolved in acetic acid (150 c.c.), and ether (600 c.c.) was added. This caused the formation of a gelatinous precipitate which became granular after a little water had been added and the mixture left overnight. It was filtered off and washed with ether. On ignition the precipitate gave a considerable residue of zinc oxide, but after several crystallisations from acetic acid the ash content was negligible. A final recrystallisation from acetic acid gave a white micro-crystalline powder (2 g.), m. p. 335—340° (decomp.) (Found: C, 59·5; H, 5·1; N, 15·2. $C_{17}H_{14}O_3N_4,CH_3{\cdot}CO_2H$ requires C, 59·7; H, 4·7; N, 14·7%). The acetic acid of crystallisation appears to be lost only just below the point of decomposition. The *product* exhibits a strong blue-white fluorescence in ultra-violet light. A solution of the material in excess of 2N-sodium carbonate was treated with powdered potassium permanganate until the liquid became permanently coloured; the mixture became slightly warm during this reaction. After saturation with sulphur dioxide the liquid was extracted with ether and the dried extract was evaporated. The residue had m. p. 120° undepressed by admixture with benzoic acid.

Chlorination of the condensation product. A mixture of (II; R = H) (8 g.) and phosphorus oxychloride (40 c.c.) was heated under reflux in a glycerol-bath. After a few minutes' gentle boiling all the solid had dissolved and a light brown solution had formed; heating was continued for a further ½ hour. The cooled liquid was poured on ice and the whole was extracted with ether. The dried ether extract was evaporated, and the residue (2 g.) was crystallised from methanol, in which it was only moderately soluble. After a further crystallisation from methanol the *chloro*-compound formed small colourless needles, m. p. 255—256° [Found: N, 15·9; Cl, 19·8; *M* (Rast), 375. $C_{17}H_{12}ON_4Cl_2$ requires N, 15·6; Cl, 19·7%; *M*, 349].

The chloro-compound (0·5 g.) was heated with piperidine (2 c.c.) for a few minutes, and the mixture was then diluted with aqueous methanol. The crystalline solid which had formed was collected and dried. It was recrystallised by dissolving it in hot benzene and then adding methanol in order to obtain a supersaturated solution; slow cooling of this solution gave large faintly yellow lozenge-shaped prisms of the *dipiperidino*-compound, m.p. 249—251° [Found: C, 70·7; H, 6·9; N, 17·8; *M* (Rast), 430. $C_{27}H_{32}ON_6$ requires C, 71·0; H, 7·0; N, 18·4%; *M*, 456].

5-*Nitro*-4-*styryluracil* (III; R = H).—5-Nitro-4-methyluracil (20 g.), benzaldehyde (80 c.c.), and piperidine (70 c.c.) were heated at first on a water-bath until the mixture had thickened and then for ½ hour in an oil-bath at 150°. The cooled mixture was diluted with methanol (200 c.c.) and the crystalline solid was collected and washed with a little methanol and then with ether. Yield of dried material, 33 g. The *piperidine* salt crystallises from hot water in long yellow prismatic needles, m. p. 236° (Found: C, 59·2, 59·2; H, 5·9, 5·4. $C_{13}H_9O_4N_3,C_5H_{11}N$ requires C, 59·3; H, 5·8%).

5-Nitro-4-methyluracil also forms a *piperidine* salt which crystallised from ethanol in yellow prisms, m. p. 207° (Found: C, 47·0; H, 6·4. $C_5H_5O_4N_3,C_5H_{11}N$ requires C, 46·9; H, 6·3%).

The salt of the styryl compound was dissolved in dilute potassium hydroxide solution (1 l.). On the addition of excess of hydrochloric acid to the warm solution, a light yellow powder was precipitated; this was collected and washed first with methanol and then with ether. The 5-*nitro*-4-*styryluracil* thus obtained decomposed on heating to 312—314° (Found: C, 55·6; H, 3·3. $C_{13}H_9O_4N_3$ requires C, 55·6; H, 3·5%).

Chloro-compound (IV). 5-Nitro-4-styryluracil (200 mg.), phosphorus oxychloride (1·0 c.c.), and dimethylaniline (0·5 c.c.) were heated under reflux for ½ hour. The mixture was poured on ice and the solid formed was collected and ground under methyl alcohol and again collected. On recrystallisation from benzene–methanol the *chloro*-compound (IV) formed fluffy yellow needles, m. p. 145—147°, and on one occasion clusters of plates, m. p. 148—149° (Found: C, 62·3; H, 4·1. $C_{13}H_{11}O_3N_4Cl$ requires C, 62·3; H, 4·1%). The dimethylaniline used in this preparation had been specially purified by the method of

Brand and Kranz (*J. pr. Chem.*, 1927, **115**, 143) which should have removed all the methylaniline. No less than 18% of methylaniline must have remained if the formation of the methylanilino-compound (IV) took place without the elimination of a methyl group from dimethylaniline.

The chloro-compound (IV) readily reacts with piperidine to give the 2-*piperidino*-derivative, m. p. 200—201° (Found: C, 69·6; H, 6·0. $C_{24}H_{25}O_2N_5$ requires C, 69·4; H, 6·1%).

2 : 6-*Dichloro*-5-*nitro*-4-*styrylpyrimidine*.—5-Nitro-4-styryluracil (2·5 g.) was heated in a sealed tube at 170° with phosphorus oxychloride (25 c.c.). The tube, held vertical, was heated in a glycerol-bath with the level of the liquid in the tube just above the level of the glycerol. The convection currents set up are sufficient to mix the contents of the tube, and there is no need for the constant rotation recommended in a similar case (Gabriel and Colman, *Ber.*, 1901, **34**, 1242). When the heating had been continued for 2 days the liquid began to darken, and about 3 hours after this the solid had completely dissolved. In other experiments the time before the solution was complete varied, being generally shorter when freshly distilled phosphorus oxychloride was used. After cooling, which caused the separation of part of the *dichloro*-compound in glistening yellow plates, the contents of the tube were poured on ice and the light brown solid was collected. A brief wash with methanol removed most of the colour, and the yellow solid was recrystallised from benzene–methanol; it formed colourless plates (2·2 g.), m. p. 200—201° (Found: C, 48·8; H, 2·3; Cl, 24·2. $C_{12}H_7O_2N_3Cl_2$ requires C, 48·7; H, 2·4; Cl, 23·9%).

5-*Nitro*-2 : 6-*dipiperidino*-4-*styrylpyrimidine*. On addition of piperidine (2 c.c.) to the powdered dichloro-compound (0·5 g.) a vigorous reaction occurred. The mixture was finally heated over a free flame for 5 minutes, by which time a paste containing piperidine hydrochloride had formed. The mixture was diluted with aqueous methanol and warmed to complete solution. On cooling and scratching the sides of the vessel a crystalline precipitate formed. The *dipiperidino*-compound crystallised from methanol in short yellow needles, m. p. 145—146° (Found: C, 66·9; H, 7·0. $C_{22}H_{27}O_2N_5$ requires C, 67·2; H, 6·9%).

2-*Chloro*-5-*nitro*-6-*amino*-4-*styrylpyrimidine* (V). The dichloro-compound (4·4 g.) was shaken with methanolic ammonia (90 c.c., saturated at room temperature). After a short time the colourless plates of the original compound were replaced by a fine yellow powder. Shaking was continued for 3 hours to ensure complete reaction, and the bright yellow solid was collected and washed with methanol and ether; yield 3·4 g. 2-*Chloro*-5-*nitro*-6-*amino*-4-*styrylpyrimidine* crystallised from a large volume of methanol in yellow plates, m. p. 245—246° (Found: C, 51·8; H, 3·8. $C_{12}H_9O_2N_4Cl$ requires C, 52·1; H, 3·3%). The same product is obtained if the reaction is carried out in boiling methanol.

When the chloroamine was treated with piperidine as already described, 5-*nitro*-2-*piperidino*-6-*amino*-4-*styrylpyrimidine*, m.p. 177—178°, was formed (Found: C, 62·6; H, 6·3. $C_{17}H_{19}O_2N_5$ requires C, 62·7; H, 5·9%).

2-*Chloro*-5 : 6-*diamino*-4-*styrylpyrimidine*. A mixture of the chloronitro-amine (V) (2·5 g.), stannous chloride crystals (12·5 g.), and hydrochloric acid (12·5 c.c., *d* 1·20) was vigorously shaken in a stoppered flask. Heat was developed and the contents of the flask which were originally fluid soon set solid. After 10 minutes' heating on a steam-bath, water was added followed by excess of sodium hydroxide solution. The precipitated diamine contained tin which could not be removed by crystallisation from aqueous alcohol. The product was therefore purified by extracting the dried material in a Soxhlet with ether—this is a rather lengthy process but it effectively removes all but traces of tin. The ether extracts, which exhibited a strong yellow-green fluorescence in daylight, yielded a yellow crystalline solid on evaporation. The diamine was further purified by dissolving it in methanol and then adding a few drops of fuming hydrochloric acid which precipitated a deep yellow hydrochloride. This was collected and suspended in fresh methanol; ammonia solution was added and the mixture was boiled. The small amount of insoluble material was filtered off, and on cooling the filtrate deposited light yellow prismatic plates (1·2 g.), m. p. 245—246°. On recrystallisation from aqueous methanol 2-*chloro*-5 : 6-*diamino*-4-*styrylpyrimidine* had m. p. 246—247° (Found in a specimen dried at 100°/0·001 mm.: C, 58·3; H, 4·4. $C_{12}H_{11}N_4Cl$ requires C, 58·4; H, 4·5%). A resinous product was obtained when the chlorodiamine was treated with piperidine.

Action of Ammonia on the Chlorodiamine.—(*a*) The chlorodiamine (1 g.) was heated with methanolic ammonia (20 c.c., saturated at room temperature) in a sealed tube at 200—210° for 5 hours. After cooling, the tube was opened and the clear yellow solution was evaporated until crystals formed. The product had m. p. 242—244° undepressed by admixture with a specimen of the original substance of m. p. 245°.

(*b*) The chlorodiamine (0·7 g.), sodium iodide (0·5 g.), and methanolic ammonia (15 c.c.) were heated in a sealed tube at 210° for 16 hours. No crystalline product could be obtained.

2-*Chloro*-8-*hydroxy*-4-*styrylpurine*.—The chloro-diamine (200 mg.) and urea (250 mg.) were intimately mixed and heated at 170—180° in an oil-bath. After ½ hour the mixture, which was fluid when first heated, had resolidified. The product was extracted with hot water and the insoluble material was collected and dissolved in dilute sodium hydroxide solution. 2-*Chloro*-8-*hydroxy*-4-*styrylpurine* was precipitated when this solution was added dropwise to boiling dilute hydrochloric acid. It was obtained as a light buff-coloured solid, decomposing when heated above 400° (Found: C, 57·5; H, 3·7. $C_{13}H_9ON_4Cl$ requires C, 57·3; H, 3·4%). The purine derivative is sparingly soluble in hot water and boiling acetic acid; a dilute solution in 2N-sodium hydroxide exhibits a strong yellow-green fluorescence, and more concentrated solutions are orange.

2 : 8 : 9-*Trihydroxy*-6-*styrylpteridine* (VI).—2-Chloro-5 : 6-diamino-4-styrylpyrimidine (1 g.) was mixed with powdered oxalic acid (4 g.) and slowly heated in an oil-bath. Considerable frothing occurred at first, but this subsided later and the temperature was then raised to 170° and kept there for 1 hour, by which time much of the unreacted oxalic acid had sublimed. The cooled product was dissolved in dilute sodium hydroxide and the solution was filtered into excess of boiling dilute hydrochloric acid. The light brown solid which separated was filtered off from the hot solution, washed with hot water, and dried at 140°/0·001 mm. The *pteridine* decomposed at 360° (Found: C, 58·9; H, 3·8. $C_{14}H_{10}O_3N_4$ requires C, 59·5; H, 3·6%).

Action of Ammonia on 2-Chloro-5-nitro-6-amino-4-styrylpyrimidine.—(*a*) The chloro-compound (V) (800 mg.) was heated with methanolic ammonia (20 c.c., saturated at room temperature) in a sealed tube at 220° for 7 hours. The contents of the tube were evaporated to half bulk and cooled; the brown granular solid which separated was collected and dissolved in dilute hydrochloric acid. The solution was decolorised by treatment with charcoal, and on the subsequent addition of ammonia a white solid was precipitated. Recrystallisation of the product from methanol gave colourless prisms, m. p. 230—231° (Found: C, 35·0; H, 4·3. Calc. for $C_5H_7O_2N_5$: C, 35·4; H, 4·2%). Gabriel and Colman (*Ber.*, 1901, **34**, 1255) give m. p. 232—233° for 5-nitro-2 : 6-diamino-4-methylpyrimidine.

(*b*) A similar experiment at 170° for 16 hours also gave the methylpyrimidine.

(*c*) A suspension of the chloro-compound (1 g.) in a saturated methanolic ammonia (20 c.c.) was heated in a sealed tube at 100—110° (glycerol-bath temperature) for 2 hours. The suspended solid gradually dissolved, and an orange solution formed. Slow cooling gave a crystalline deposit (rapid cooling causes the formation of gelatinous material). The product (0·5 g.) was collected and recrystallised by dissolving it in acetone, then adding methanol, and evaporating until crystals formed. On cooling, *5-nitro-2 : 6-diamino-4-styrylpyrimidine* separated in yellow prisms, m. p. 227—229° (Found: C, 55·8; H, 4·3. $C_{12}H_{11}O_2N_5$ requires C, 56·0; H, 4·3%).

2 : 5 : 6-*Triamino-4-styrylpyrimidine* (VIII; R = H).—The above nitro-diamine (0·9 g.), stannous chloride crystals (4·5 g.), hydrochloric acid (10 c.c., *d* 1·20), and methanol (10 c.c.) were heated on a steam-bath. The prismatic crystals of the nitro-diamine gradually dissolved and were replaced by a granular orange solid. After ½ hour's heating the mixture was cooled in ice and the stannichloride complex (2 g.) was filtered off. The orange solid was suspended in methanol and concentrated aqueous ammonia solution was added dropwise until the solid had dissolved. The gelatinous precipitate which formed was filtered off and the filtrate was evaporated to small bulk; a crop of small yellow prisms was thus obtained. The *monohydrochloride* of the triamine thus obtained had m. p. >280° (decomp.) (Found: C, 54·6, 54·6; H, 5·5, 5·4. $C_{12}H_{13}N_5$,HCl requires C, 54·7; H, 5·4%).

Addition of a slight excess of sodium hydroxide to a solution of the monohydrochloride in water precipitated the *triamine*. Recrystallisation of the base from aqueous methanol gave flattened yellow needles, m. p. 218—220° (Found: C, 63·0; H, 5·75; N, 30·2. $C_{12}H_{13}N_5$ requires C, 63·4; H, 5·8; N, 30·8%).

2-Amino-8-hydroxy-4-styrylpurine.—The triamine (VII) (300 mg.) and urea (1 g.) were heated at 150° in an oil-bath. A clear brown fluid formed at first, but this gradually solidified and ammonia was evolved. After 1 hour's heating the product was cooled and dissolved in dilute sodium hydroxide solution, and this solution was filtered into excess of boiling dilute hydrochloric acid. No solid separated, and so sodium acetate was added until a yellow amorphous powder was deposited. This was the *purine monohydrate*; it had m. p. 345° (slight decomp.) (Found: C, 57·5; H, 4·8. $C_{13}H_{11}ON_5$,H_2O requires C, 57·6; H, 4·8%).

4-Styryl-4-deoxyleucopterin.—A mixture of powdered oxalic acid (3 g.) and the triamine (1 g.) was heated gradually to 160° in an oil-bath. Much frothing occurred in the early stages but this subsided after 2 hours' heating. The cooled product was dissolved in excess of 2N-sodium hydroxide—the sodium salt of the substance is somewhat sparingly soluble—and the solution was filtered into boiling dilute hydrochloric acid. The light buff-coloured precipitate was unfilterable and so it was collected and washed with water in a centrifuge tube. The *pterin* derivative was dried by washing it with methanol and then drawing hot air through the centrifuge tube. The compound decomposed above 380° (Found: C, 56·1; H, 4·1; N, 23·4. $C_{14}H_{11}O_3N_5$,H_2O requires C, 56·2; H, 4·4; N, 23·4%). A dilute solution of the compound in 2N-sodium hydroxide exhibits a strong yellow fluorescence in ultra-violet light. Addition of concentrated sodium hydroxide solution to this solution precipitates a gelatinous sodium salt which becomes granular on standing.

Reaction between the Triamine and Glyoxylic Acid.—The triamine (VII; R = H) (0·5 g.), barium glyoxylate (1 g.), and sulphuric acid (12 c.c., 70%) were heated for 1 hour on a steam-bath. The mixture was diluted with an equal volume of water and excess of ammonia was added. Barium sulphate was removed by centrifuging the suspension, and the supernatant liquid was neutralised with acetic acid. The brown precipitate was collected and washed first with water and then with methanol in a centrifuge tube. The dried material still had a considerable ash content; its solution in dilute ammonia or 2N-sodium hydroxide showed a yellow-green fluorescence resembling that of similarly prepared xanthopterin solutions.

Action of Mesoxalic Ester on the Triamine (VII; R = H).—To a solution of the triamine (300 mg.) in hydrochloric acid (100 c.c., 2N) was added a solution of mesoxalic ester hydrate (1 g. in 10 c.c. of water), and the mixture was heated for 4 hours on a steam-bath. When the solution cooled a dark orange granular solid separated; this was collected and washed with a little water. The acidic *product* was purified by adding its solution in dilute alkali to boiling 2N-hydrochloric acid. On cooling, the solution deposited an orange solid which still retained some sodium salts; it decomposed above 360° (Found: N, 20·2. $C_{15}H_{11}O_3N_5$,H_2O requires N, 21·4%).

Condensation of p-*Nitrobenzaldehyde with 4-Methyluracil.*—Methyluracil, *p*-nitrobenzaldehyde, and powdered anhydrous zinc chloride (5 g. of each) were mixed and heated at 150—160° for 1 hour, and the temperature was then raised to 170—180° for 3 hours, the mass being stirred occasionally. After cooling, the product was ground under methanol and then collected. The dark brown solid was dissolved in boiling glacial acetic acid; the filtered solution deposited lighter coloured material on cooling (2·3 g.). It was further purified by dissolution in dilute alkali followed by reprecipitation by adding the solution dropwise to boiling 2N-hydrochloric acid. After two more crystallisations from acetic acid the *product* formed small light yellow plates, decomposing above 275° (Found, in a specimen dried at 120°/0·01 mm.: C, 52·8; H, 4·2; N, 16·0. $C_{17}H_{13}O_5N_5$,$CH_3{\cdot}CO_2H$ requires C, 53·4; H, 4·0; N, 16·4%).

Chlorination of the condensation product (II; R = NO_2). A mixture of (I) (2 g.) and phosphorus oxychloride (20 c.c.) was heated in a sealed tube in a glycerol-bath, the temperature being raised gradually to 170° over a period of 1½ hours, during which time the solid completely dissolved. The contents of the

tube were poured on ice, and after decomposition of the excess of reagent the insoluble buff-coloured solid was collected, washed with a little methanol, and dried. The *dichloro*-compound (1 g.) separated from hot acetone as a microcrystalline powder, m. p. 270° (Found: N, 18·0; Cl, 17·0. $C_{17}H_{11}O_3N_5Cl_2$ requires N, 17·3; Cl, 17·5%).

The chloro-compound (0·5 g.) was treated with piperidine (1 g.); heat developed and a crystalline mass was formed. After five minutes' heating over a free flame the mixture was diluted with ethanol and water was added until crystals formed. When recrystallised from acetone the *dipiperidino*-compound formed small prisms, m. p. 275—278° [Found: C, 64·4, 65·0; H, 5·9, 5·9; N, 18·9; *M* (Rast), 475. $C_{27}H_{31}O_3N_7$ requires C, 64·7; H, 6·2; N, 19·5%; *M*, 501].

Condensation of p-*nitrobenzaldehyde with* 5-*nitro*-4-*methyluracil*.—(*a*) Nitromethyluracil (8·5 g.), *p*-nitrobenzaldehyde (7·5 g.), and piperidine (20 c.c.) were mixed and heated at 160° for ½ hour. After cooling and diluting with methanol the product was collected and recrystallised from methanol, forming reddish prisms, m. p. 202—205°. This material was suspended in hot water and treated with excess of hydrochloric acid; the cooled solution deposited pale yellow prisms of unchanged nitromethyluracil, m. p. 272° (decomp.) (Found: C, 35·1; H, 3·1. Calc. for $C_5H_5O_4N_3$: C, 35·1; H, 2·9%).

(*b*) Nitromethyluracil (500 mg.), nitrobenzaldehyde (500 mg.), and ethanol (6 c.c.) containing concentrated hydrochloric acid (3 c.c.) were heated under reflux for 3 hours. Only unchanged nitromethyluracil was isolated.

(*c*) A mixture of nitromethyluracil, nitrobenzaldehyde, and powdered anhydrous zinc chloride (10 g. of each) was heated in a glycerol-bath at 150° for 3 hours. The product was ground under methanol and collected, then heated with water, and the hot suspension was filtered rapidly to remove any unchanged nitromethyluracil. The solid was not appreciably soluble in dilute alkali and so it was purified by dissolution in hot aqueous piperidine. On cooling, this solution deposited bright yellow needles (12 g.) of the *piperidine* salt, m. p. 235° (decomp.) (Found: C, 52·8; H, 5·0. $C_{17}H_{18}O_6N_5$ requires C, 52·4; H, 4·9%). The salt was suspended in methanol and on the addition of excess of hydrochloric acid a white granular solid separated. This was filtered off and washed successively with methanol, water, methanol, and ether. 5-*Nitro*-4-p-*nitrostyryluracil* (III; R = NO_2) decomposed above 300° (Found: C, 47·5; H, 2·6. $C_{12}H_8O_6N_4$ requires C, 47·4; H, 2·7%).

2 : 6-*Dichloro*-5-*nitro*-4-p-*nitrostyrylpyrimidine*.—The nitro-compound (II) (2 g.) was heated with phosphorus oxychloride in a sealed tube at 170—180° for 3 days, by which time all the solid had dissolved to give a dark brown solution. When the solution was cooled yellow needles were deposited and the mixture was poured on ice. After ½ hour the mixture was filtered. The light brown solid was crystallised from acetone, forming long colourless needles of the *dichloro*-compound, m. p. 218—220° (Found: C, 42·3; H, 2·0. $C_{12}H_6O_4N_4Cl_2$ requires C, 42·3; H, 1·8%).

Action of Ammonia on 2 : 6-*Dichloro*-5-*nitro*-4-p-*nitrostyrylpyrimidine*.—(*a*) The dichloro-compound (500 mg.) was heated under reflux with saturated methanolic ammonia (20 c.c. for ½ hour). The suspended solid was collected and washed with methanol; it had m. p. 218°, undepressed by admixture with starting material.

(*b*) The dichloro-compound (1 g.) was heated with methanolic ammonia (20 c.c., saturated at room temperature) in a sealed tube at 110° for 1½ hours. A red colour developed and the solid appeared to react without passing into solution—the crystals crumbled to a fine powder—and the tube was occasionally inverted cautiously to mix the contents. After cooling, the solid was collected and washed with a little methanol; yield, 0·6 g. The product crystallised from acetic acid as colourless felted needles; these probably contained solvent of crystallisation, for on heating they became yellow, and on warming with methanol they were converted into a granular yellow powder. 5-*Nitro*-2 : 6-*diamino*-4-p-*nitrostyrylpyrimidine* crystallised from a large volume of methanol in yellow micro-crystals, m. p. 286° (Found: C, 48·3; H, 3·4. $C_{12}H_{10}O_4N_6$ requires C, 47·7; H, 3·3%).

5-*Nitro*-2 : 6-*dipiperidino*-4-p-*nitrostyrylpyrimidine*. The dichloro-compound (200 mg.) was warmed on steam with piperidine (0·5 c.c.) for ½ hour, and then water was added and the precipitated solid was collected, washed with methanol, and dried. The product was dissolved in hot benzene, and methanol was added until crystals formed; on cooling, the *dipiperidino*-compound separated as a yellow microcrystalline powder. Recrystallisation from benzene–methanol gave short yellow needles, m. p. 230—231° (Found: C, 60·7; H, 5·8. $C_{22}H_{26}O_4N_6$ requires C, 60·3; H, 6·0%).

2 : 5 : 6-*Triamino*-4-p-*aminostyrylpyrimidine* (VII; R = NH_2).—5-Nitro-2 : 6-diamino-4-*p*-nitrostyrylpyrimidine (2 g.) was suspended in hydrochloric acid (20 c.c. *d* 1·20), stannous chloride crystals were added, and the mixture was shaken vigorously. It is essential to add the reactants in this order, for on one occasion when the two solids were mixed dry a very vigorous reaction soon started and the mass charred. Heat was developed during the shaking, and after the initial reaction had subsided the mixture was heated for 10 minutes on a steam-bath. After cooling, the stannichloride complex was filtered off and dissolved in water. Hydrogen sulphide was passed into the solution until no more tin was precipitated. The filtered solution was evaporated almost to dryness, methanol was added then, and the solvent was removed on a steam-bath. The orange-red residue was heated with dilute sodium hydroxide solution and the small amount of insoluble material was filtered off. The cooled filtrate yielded clusters of long flattened needles, m. p. 222—224°. The *tetra-amine* was recrystallised by first dissolving it in methanol—in which it is much more soluble than in water—and then adding water and carefully removing some of the alcohol by evaporation; the product had m. p. 223—224° (Found: C, 59·6, 59·2; H, 5·9, 5·8. $C_{12}H_{14}N_6$ requires C, 59·4; H, 5·8%). When fuming hydochloric acid is added to a solution of the base in methanol the hydrated *hydrochloride* is precipitated as an orange micro-crystalline powder which decomposes above 290° (Found: C, 47·5; H, 5·7. $C_{12}H_{14}N_6,HCl,1\frac{1}{2}H_2O$ requires C, 47·1; H, 5·9%).

Reaction between Oxalic Acid and the Tetra-amine (VII; R = NH_2).—The tetra-amine (500 mg.) and powdered oxalic acid (1 g.) were intimately mixed in a wide test tube and heated gradually in an oil-bath. After 2 hours at 140° the cooled chocolate-coloured melt was warmed with water to remove unchanged base and oxalic acid. The brown solid was collected and dried; it decomposed without

melting when heated (Found : C, 49·4; H, 4·3; N, 21·7%). The product was dissolved in a large volume of dilute alkali to give a yellow solution, and when more concentrated alkali was added a light yellow sodium salt was precipitated; the yellow ammonium salt is also sparingly soluble in water. When the sodium salt was heated for a short time with dilute alkali it was recovered unchanged; after prolonged heating with 4N-sodium hydroxide a small amount of the original tetra-amine could be isolated.

Reaction between Urea and the Tetra-amine (VII; R = NH_2).—Urea (1 g.) and the tetra-amine (500 mg.) were mixed and heated gradually to 170°. The cooled product was heated with water, and the insoluble material was collected and dissolved in hot 4N-sodium hydroxide—it is not appreciably soluble in cold dilute alkali. Addition of acetic acid caused the formation of a gelatinous precipitate; this was washed twice with water and twice with methanol in a centrifuge tube. The dried material still contained about 4% of ash; it decomposed without melting when heated [Found : C, 46·3; H, 5·1; N, 20·8% (corrected for ash content)].

Condensation of p-*Dimethylaminobenzaldehyde with* 5-*Nitro*-4-*methyluracil*.—A mixture of *p*-dimethylaminobenzaldehyde (3·0 g.), nitromethyluracil (3·4 g.), and piperidine (10 c.c.) was heated at 160° (glycerol-bath temperature) for ½ hour and then cooled and diluted with methanol. The sparingly soluble *piperidine* salt which separated was heated with this solvent and collected; it formed brick-red crystals which decomposed on heating above 300° (Found : C, 59·3; H, 6·7. $C_{14}H_{14}O_4N_4.C_5H_{11}N$ requires C, 58·9; H, 6·5%). The salt dissolved in dilute hydrochloric acid, to give a purple solution which soon became colourless, and when excess of sodium acetate was added to the hot solution 5-*nitro*-4-p-*dimethylaminostyryluracil* (III; R = NMe_2) was obtained as a crimson amorphous precipitate which had m. p. 285—290° (decomp.) (Found : C, 55·3; H, 4·8. $C_{14}H_{14}O_4N_4$ requires C, 55·6; H, 4·7%).

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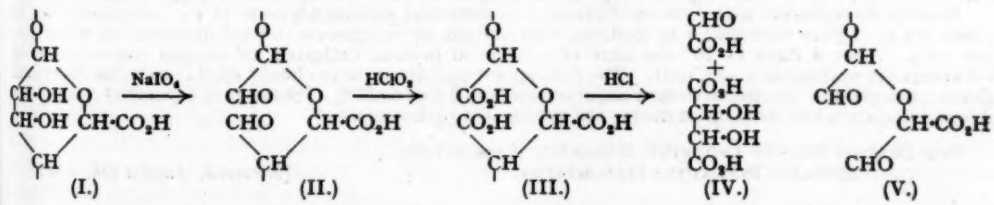
225. *Formation of Tartaric Acid by Oxidation and Hydrolysis of Oxycelluloses produced by Dinitrogen Tetroxide.*

By FRANK S. H. HEAD.

Kenyon and his co-workers concluded that dinitrogen tetroxide oxidises the primary alcohol groups in cellulose to carboxyl groups. Evidence in support of this has been obtained by oxidising a "dinitrogen tetroxide oxycellulose" with sodium metaperiodate to (II), converting (II) into (III) with chlorous acid, hydrolysing (III), and isolating *meso*tartaric acid.

THE oxidation of cellulose with dinitrogen tetroxide has been studied by Kenyon and his co-workers (*J. Amer. Chem. Soc.*, 1942, **64**, 121, 127; 1947, **69**, 342, 347, 349, 355), who concluded that the primary alcohol groups in the cellulose were preferentially attacked and converted into carboxyl groups of uronic type. A similar conclusion was reached by Maurer and Reiff (*J. Makromolekulare Chem.*, 1943, **1**, 27). "Dinitrogen tetroxide oxycelluloses" accordingly contain units of the structure (I), and it is to be expected that such units would be oxidised by sodium metaperiodate to structural units (II), and further by chlorous acid to units (III); these should be hydrolysed by acid to glyoxylic and tartaric acids (IV). By this series of reactions it has been possible to obtain *meso*tartaric acid, isolated as the acid brucine salt, from a "dinitrogen tetroxide oxycellulose" with an over-all yield of 28%. *meso*Tartaric acid could only be formed among the hydrolysis products if the primary alcohol group in some of the structural units of the original oxycellulose had already been oxidised, though its presence would naturally be expected whether this alcohol group had been oxidised to aldehyde or to carboxyl.



Lucas and Stewart (*J. Amer. Chem. Soc.*, 1940, **62**, 1792), for another purpose, carried out a similar series of transformations with alginic acid, and a much modified version of their method of working was adopted. The oxidation of "dinitrogen tetroxide oxycellulose" with sodium metaperiodate was progressive and there was no sign of a break in the rate of oxidation when

one mole of oxidant had been consumed. This may be connected with the instability to periodate of terminal units, such as (V), containing reactive methylene groups (cf. Huebner, Ames, and Bubl, *J. Amer. Chem. Soc.*, 1946, **68**, 1621), and to the consequent progressive oxidation of the chains from one end.

The oxidation was discontinued at an arbitrarily selected stage. Lucas and Stewart heated the hydrolysate (IV) with baryta to convert the glyoxylic acid into barium glycollate (soluble) and oxalate, and separated oxalic acid from tartaric acid as the copper salt. The latter procedure was found to be superfluous, for brucine oxalate is much more soluble than brucine hydrogen *meso*tartrate.

Application of the modified procedure to sodium alginate oxidised with sodium metaperiodate gave a 27% yield of brucine hydrogen *meso*tartrate.

Experimental,

Brucine Hydrogen meso*Tartrate*.—The authentic salt prepared according to Lucas and Stewart (*loc. cit.*) consisted of colourless needles, $[\alpha]_D^{20°}$ − 22·5° (*c*, 0·5 in water), the m. p. of which varied considerably with the initial temperature of the oil-bath; *e.g.*, for a rate of heating of about 6° per min. and an initial temperature of 200° the m. p. (decomp.) was 252°, but with an initial temperature of 250° it was 258°. Lucas and Stewart quote m. p. 259° (uncorr.) and $[\alpha]_D^{20°}$ − 23° (*c*, 0·5).

" Dinitrogen Tetroxide Oxycellulose."—The material used was prepared by treating dry, scoured Texas cotton (10 g.) with gaseous dinitrogen tetroxide (16·94 g.) at 20° for 8 days. It contained 11·0% of moisture and 0·78 carboxyl group per C_6 unit as determined by the calcium acetate method (Lüdtke, *Biochem. Z.*, 1934, **268**, 372).

Oxidation of " Dinitrogen Tetroxide Oxycellulose " with Sodium Metaperiodate. Production of (II).—(a) The oxycellulose (0·96 g.) was suspended in a solution of sodium metaperiodate (2·15 g.) in water (100 c.c.) and the whole was kept at 20°. 10-C.c. portions of the liquor were withdrawn at intervals and the excess periodate present was determined by the method of Müller and Friedberger (*Ber.*, 1902, **35**, 2652). The figures for oxygen consumed after various times were :

Time, days	1	2	3
Oxygen consumed, atoms per C_6 unit ...	1·00	1·42	1·62

(b) The oxycellulose (5·00 g.) was suspended in a solution of sodium metaperiodate (10·70 g.) in water (500 c.c.) and the whole was kept at 20° for 48 hours. 20 C.c. were withdrawn for titration as above and the oxygen consumption figure was found to be 1·59. The originally fibrous oxycellulose was completely dissolved or dispersed during the oxidation, the reaction product being a turbid solution from which nothing was precipitated on acidification. The solution was treated with a small excess of potassium iodide (43 g.) and with excess of hydrochloric acid. Iodine was removed by filtration and extraction of the filtrate with methylene chloride. The liquor was neutralised to Congo-red by addition of sodium hydroxide.

Conversion of (II) *into* (III).—Sodium chlorite (15·5 g.) was dissolved in the above solution, glacial acetic acid (15 c.c.) added, and the whole kept at 20° for 20 hours. Excess of chlorite was destroyed by adding 40% formaldehyde (15 c.c.) and the solution was freed from chlorine dioxide by aeration.

Hydrolysis of (III) *and Isolation of* meso*Tartaric Acid*.—The above solution was made acid to Congo-red by addition of hydrochloric acid (20 c.c.) and heated on the steam-bath for 16 hours. It was then made just alkaline by addition of barium hydroxide and heated for a further 3 hours. The precipitate of insoluble barium salts was collected, well washed, and dried at 110° (4·0 g.). It was powdered, suspended in a little water containing the theoretical amount (28·3 c.c.) of N-sulphuric acid (assuming precipitate to be barium tartrate), heated on the steam-bath for a short time, and filtered. The filtrate (90 c.c.) was halved. Half was treated with alcohol (45 c.c.) and made alkaline to litmus by adding an excess of brucine. The second half was then added, and the mixture was heated on the steam-bath for 3 hours, diluted with water (150 c.c.), cooled, and extracted three times with chloroform. The aqueous portion was concentrated to about 35 c.c. on the steam-bath and left over-night. Brucine hydrogen *meso*tartrate separated as colourless needles, m. p. 252°.* The yield (3·11 g., dried at 110°) corresponded to 28% of the theoretical, assuming that the original oxycellulose was a polymer containing 78% of anhydroglucuronic acid units, the remainder being anhydroglucose units. Recrystallisation gave material of m. p. 257° * which did not depress the m. p. of an authentic specimen; $[\alpha]_D^{20°}$ − 22° (*c*, 0·5).

Parallel Experiments with Sodium Alginate.—Commercial sodium alginate (4 g.; corresponding to about 3·4 g. of pure material) was oxidised with sodium metaperiodate (6·6 g.) dissolved in water (*ca.* 250 c.c.). After 4 days at 20° the titre of a weighed portion indicated an oxygen consumption of 1·6 atoms per mannuronic acid unit. The subsequent operations were closely similar to those described above; the yield of brucine hydrogen *meso*tartrate was 2·5 g. or 27%. Some gum separated during the chlorite oxidation but redissolved during the subsequent hydrolysis.

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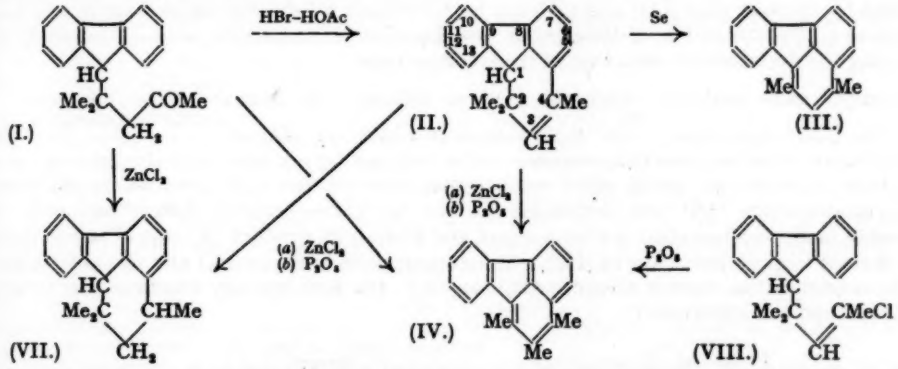
* Uncorrected m. p.s determined with an initial bath temperature of 250°.

226. *The Condensation of Fluorene with Acetone. Part IV. Synthesis of* 2 : 4-*Dimethylfluoranthene.*

By James Forrest and S. Horwood Tucker.

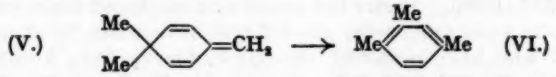
2 : 2 : 4-Trimethyl-1 : 2-dihydrofluoranthene (II) has been converted by dehydrogenation with selenium into 2 : 4-dimethylfluoranthene (III), and by heating with phosphoric oxide into 2 : 3 : 4-trimethylfluoranthene (IV). The migration of methyl from *gem*-dimethylated compounds is discussed. (III) has been synthesised from 1 : 3-dimethylnaphthalene, and an attempt made to synthesise (IV) starting from fluorenone-1-carboxylic acid.

The reaction of fluorene and acetone in presence of potassium hydroxide gives rise to methyl 2-(9-fluorenyl)-2-methyl-*n*-propyl ketone (I) [Maitland and Tucker, *J.*, 1929, 2559 (Part I); France, Maitland, and Tucker, *J.*, 1937, 1739 (Part II)] which can be readily transformed by the action of hydrogen bromide in glacial acetic acid into the fluoranthene derivative, 2 : 2 : 4-trimethyl-1 : 2-dihydrofluoranthene (II) [France, Tucker, and Forrest, *J.*, 1945, 7 (Part III)]. This substance undergoes interesting reactions: (*a*) selenium at 300° for 5 hours removes hydrogen and methyl with production of 2 : 4-*dimethylfluoranthene* (III); (*b*) phosphoric oxide at 250° for 90 minutes brings about migration of a methyl group of (II), with dehydrogenation, to give 2 : 3 : 4-*trimethylfluoranthene* (IV); (*c*) (IV) is also obtained, together with 2 : 2 : 4-trimethyl-1 : 2 : 3 : 4-tetrahydrofluoranthene (VII), when (II) is heated with anhydrous zinc chloride at 250° for 4 hours.



Methyl groups are readily eliminated during dehydrogenation processes when they are attached to quaternary carbon, as, *e.g.*, when in angle positions or in *gem*-dimethyl groups (Linstead and Thomas, *J.*, 1940, 1127; *Ann. Reports*, 1936, **33**, 294; Kloetzel, *J. Amer. Chem. Soc.*, 1940, **62**, 3405). Hence the formation of (III) from (II) by the action of selenium is to be expected, since the *gem*-dimethylated ring of (II), having potentially two double bonds already present and a readily ionisable hydrogen atom (attached to the fluorene skeleton), should aromatise readily.

On the other hand, whilst *migration* of methyl groups during dehydrogenation is rare (Linstead and Thomas, *loc. cit.*) migration (without dehydrogenation) by the action of acidic reagents is well known, as in the ready conversion of (V) into (VI) (Auwers and Ziegler, *Annalen*, 1921, **425**, 217; for electronic interpretation, see Whitmore, "Organic Chemistry", 1937, p. 808).



Formation of the aromatic compound (IV) from (II) by the action of acidic reagents is accordingly understandable. It throws light on the observation (France, Maitland, and Tucker, *loc. cit.*, p. 1744) that the ketone (I), by the action of zinc chloride or of phosphoric oxide at 250°, is transformed into a substance, m. p. 133—134°. This is now seen to be (IV), supported by the analytical figures previously found.

Whether in the conversion of the ketone (I) into the fluoranthene (IV) the aromatisation process takes place during *cyclo*-dehydration or *via* (II), intermediately formed, cannot be decided. The fact that (II) can be converted into (IV), under the same experimental conditions as those used in the conversion of (I) into (IV), shows that the latter reaction sequence, (I) ⟶

(II) ⟶ (IV), is possible. At any rate methyl migration from a quaternary carbon atom during dehydration with acidic reagents at relatively high temperature is known (see, *e.g.*, Meerwein, *Annalen*, 1914, **405**, 129; Cohen, Cook, and Hewett, *J.*, 1935, 445; Kloetzel, *loc. cit.*), and conversion of (II) into (IV) follows possibly the same pattern; but in the transformation here considered it must not be forgotten that dehydrogenation also occurs. This must at least be facilitated by the mobility of the 1-hydrogen atom, and its tendency to separate as a proton.

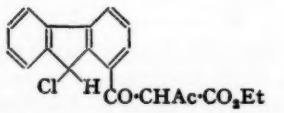
The formation of a small quantity of 2 : 2 : 4-trimethyl-1 : 2 : 3 : 4-tetrahydrofluoranthene (VII) (France, Tucker, and Forrest, *loc. cit.*) by the action of zinc chloride on (I) or (II) is clearly due to reduction of (II) [intermediately formed from (I)] by hydrogen derived from another molecule of (II) in its transformation into (IV). That it was not isolated in the corresponding reaction using phosphoric oxide may be due to oxidative action of this reagent.

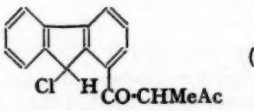
Fusion of 2-chloro-4-(9-fluorenyl)-4-methylpent-2-ene (VIII) (France, Tucker, and Forrest, *loc. cit.*) with phosphoric oxide also gave (IV).

(IV) behaves unusually in that it forms a *picrate* containing two molecules of the hydrocarbon to one of picric acid; but it gives a normal *complex* (1 : 1 mols.) with trinitrobenzene. (III) gives 1 : 1-molecular *complexes* in both cases. The dihydro- and tetrahydro-fluoranthenes do not give addition complexes.

There is evidence for fixity of the double bonds in the methylated rings of (III) and (IV), since on microhydrogenation, using palladium black (Heilbron, Sexton, and Spring, *J.*, 1929, 929) in acetic acid, the amount of hydrogen absorbed indicates the presence in each compound of two double bonds. The olefinic nature of these exo-fluorene-skeleton double bonds is also indicated by the fact that (III) and (IV) are both oxidised by sodium dichromate in acetic acid solution to a mixture of 1-acetylfluorenone and fluorenone-1-carboxylic acid—incidentally also supporting the fluoranthene structure of these compounds.

Attempts were made to synthesise (IV) as follows: fluorenone-1-carboxylic acid $\xrightarrow{\text{redn.}}$ *9-fluorenol-1-carboxylic acid* $\xrightarrow{PCl_5}$ *9-chlorofluorene-1-carboxyl chloride* $\xrightarrow{CH_3\cdot CO\cdot CMeNa\cdot CO_2Et}$ *ethyl 9-chlorofluorene-1-carbonylmethylacetoacetate* to be followed by reaction with zinc or magnesium, etc. Model experiments using ethyl sodioacetoacetate showed that ethyl 9-chlorofluorene-1-carbonylacetoacetate (IX) was obtainable (as also the corresponding *bromo*-compound); but when ethyl methylacetoacetate was employed, the hydrolysis product (X) was obtained instead of the desired methyl derivative of (IX). Since compounds of types (IX) and (X) are obviously capable of enolisation, further advance (utilising, *e.g.*, the Reformatsky reaction) was unlikely, and the method was abandoned.

(IX.) 



(X.)

Attempts are being made to synthesise 2 : 4-dimethylfluoranthene (III) from β-9-fluorenylbutyronitrile but since this follows the synthetic line herein described, starting with fluorene, it was decided to confirm the fluoranthene structure unambiguously by commencing with a naphthalene foundation. Accordingly, (III) has now been synthesised by the following outlined process: 1 : 3-dimethylnaphthalene $\xrightarrow{NaI\text{–}NaNO_2\text{–}HOAc}$ 1-iodo-2 : 4-dimethylnaphthalene $\xrightarrow{o\text{-bromonitrobenzene–Cu}}$ 1-(2-*nitrophenyl*)-2 : 4-*dimethylnaphthalene* $\xrightarrow{\text{redn.}}$ *amine* $\xrightarrow{\text{Pschorr reactn.}}$ (III) (cf. Ullmann, *Annalen*, 1904, **332**, 38; Lesslie and Turner, *J.*, 1930, 1758; 1931, 1188; Rule and Smith, *J.*, 1937, 1096). Under the conditions employed there was no evidence of the formation of 2-methyl-3 : 4-benzofluorene, which might have arisen by analogy with the work of Mascarelli and Longo who have obtained dimethylfluorenes from 2-nitro-2 : *x* : *y*-trimethyldiphenyls (*Gazzetta*, 1941, **71**, 289, 293, 297; cf. also *Atti R. Accad. Sci. Torino*, 1940—41, **76**, 66, 70).

Probably the greater reactivity of hydrogen in the α-position of the naphthalene nucleus than that in the methyl group accounts for the course taken in our fluoranthene synthesis.

As a model experiment fluoranthene was synthesised by this method. The method of preparing fluorenone-1-carboxylic acid by oxidation of fluoranthene (Fieser and Seligman, *J. Amer. Chem. Soc.*, 1935, **57**, 2174) has been improved.

(III) and (IV) have no growth-inhibitory effect (Badger, Elson, Haddow, Hewett, and Robinson, *Proc. Roy. Soc.*, 1942, *B*, **130**, 268, 270) and no carcinogenic activity (Badger, Cook, Hewett, Kennaway, Kennaway, and Martin, *ibid.*, 1942, *B*, **131**, 170).

Experimental.

Preparation of 2 : 4-*Dimethylfluoranthene* (III).—(a) *From* 2 : 2 : 4-*trimethyl*-1 : 2-*dihydrofluoranthene* (II). (II) (2 g.) mixed with selenium powder (0·7 g.) was heated at 300° for 5 hours. The product was extracted with boiling acetic acid; on cooling, faintly green plates separated and were recrystallised from ethanol, giving 2 : 4-*dimethylfluoranthene* (III), m. p. 113—115° (1·6 g.; yield, 80%). It slowly turns yellow superficially even when preserved in closed tubes (Found: C, 94·1; H, 6·3. $C_{18}H_{14}$ requires C, 93·9; H, 6·1%). Microhydrogenation of (III), using palladium in acetic acid, indicated 2·17 double bonds. (III) forms an orange *picrate* from ethanol, softening at 200°, melting at 206° (Found: C, 62·9; H, 3·9; N, 9·05. $C_{18}H_{14},C_6H_3O_7N_3$ requires C, 62·7; H, 3·7; N, 9·15%). (III) gives a *s*-trinitrobenzene *complex*, yellow needles from methanol, softening at 210°, melting at 223° (Found: C, 65·0; H, 3·8; N, 9·5. $C_{18}H_{14},C_6H_3O_6N_3$ requires C, 65·0; H, 3·8; N, 9·5%).

(b) *From* 2 : 2 : 4-*trimethyl*-1 : 2 : 3 : 4-*tetrahydrofluoranthene* (VII). (VII) (2 g.) was treated with selenium (1·3 g.) as under (a) above and similarly gave (III) (1·15 g.; yield, 60%). Similar results were obtained when sulphur was used; also a substance, which contained sulphur, was isolated as scarlet needles, m. p. > 250° (Found: C, 67·1; H, 3·3%).

Synthesis of 2 : 4-*Dimethylfluoranthene* (III).—1-*Iodo*-2 : 4-*dimethylnaphthalene.* 1 : 3-Dimethylnaphthalene (Barnett and Sanders, *J.*, 1933, 434) (5·2 g. purified by means of its picrate), sodium iodide (5 g.), and sodium nitrate (3·5 g.) were boiled in glacial acetic acid (100 ml.) until the iodine colour had disappeared (~ 1 hr.). The solution was poured into water, extracted with chloroform or carbon tetrachloride, and the washed extract distilled under reduced pressure. The fraction boiling at 139—144°/1 mm., a pale yellow oil, was retained as 1-iodo-2 : 4-dimethylnaphthalene (6·5 g.; yield 70%). The substance rapidly darkened owing to separation of iodine; accordingly, analyses were discordant.

1-(2-*Nitrophenyl*)-2 : 4-*dimethylnaphthalene.* 1-Iodo-2 : 4-dimethylnaphthalene (5 g.) and *o*-bromonitrobenzene (4 g.) were heated at 220—230°; copper bronze (6 g.) was added at intervals, and heating continued with occasional shaking for 2 hours. The mixture was finally heated at 270° for 10 minutes, then steam distilled to remove unchanged *o*-bromonitrobenzene, and the residue extracted with methanol (charcoal). On concentration of the solution, pale brown hexagonal prisms separated, and more grew during several days; the 1-(2-*nitrophenyl*)-2 : 4-*dimethylnaphthalene* crystallised from glacial acetic acid in lemon-yellow hexagonal plates, m. p. 119—120° (1·38 g.; yield, 28%) (Found: C, 77·85; H, 5·3; N, 5·2. $C_{18}H_{15}O_2N$ requires C, 78·0; H, 5·4; N, 5·0%).

1-(2-*Aminophenyl*)-2 : 4-*dimethylnaphthalene.* The nitro-compound prepared above (0·24 g.) was reduced by palladium black (0·1 g.) in glacial acetic acid (15 ml.) in 1 hour. The filtered solution, on being made alkaline with potassium hydroxide solution, gave a pale brown precipitate which was extracted with ether. Evaporation, extraction of the residue with dilute hydrochloric acid, extraction of this solution with ether, and treatment of the acid solution with alkali gave, after crystallisation from methanol diluted with a trace of water (scratching), slightly brown, thick, square-sided plates, of 1-(2-*aminophenyl*)-2 : 4-*dimethylnaphthalene*, softening at 107°, melting at 110°. The crystals contained methanol of crystallisation, and were dried in a vacuum at 70° before being analysed (0·12 g.; yield, 55%) (Found: C, 87·1; H, 6·8; N, 5·9. $C_{18}H_{17}N$ requires C, 87·4; H, 6·9; N, 5·7%).

The nitro-compound was also reduced by platinum (from PtO_2) in acetic acid; reduction was slower (4 hours) with palladium on strontium carbonate and ethanol. It was also reduced by stannous chloride in acetic acid saturated with hydrogen chloride, but the product was difficult to purify and the yield much lower than that obtained by catalytic reduction.

The *acetyl* derivative of the amine was obtained by boiling (1 minute) with acetic anhydride, and gave prisms from methanol, softening at 138°, melting at 142° (Found: C, 83·4; H, 6·6. $C_{20}H_{19}ON$ requires C, 83·1; H, 6·6%).

2 : 4-*Dimethylfluoranthene* (III). The above amine was diazotised with sodium nitrite and dilute sulphuric acid, and copper bronze added. The red solution was warmed until nitrogen evolution ceased; the solution became pale yellow (10 minutes), and brown solid aggregated about the copper powder. The solid was extracted by methanol to give a violet fluorescent solution from which there separated first oil and then crystalline nodules, or the solid was sublimed in a vacuum at 130—140°/11 mm., to give (III), which was identified by m. p., m. p. of a mixture with (III) prepared by method (a) above, and by its *s*-trinitrobenzene complex. The yield was very poor.

Synthesis of Fluoranthene.—α-Iodonaphthalene (7 g.), *o*-bromonitrobenzene (6 g.), and copper bronze (5 g.) were heated at 240—250° for 3 hours. The product, worked up as for the corresponding 2 : 4-dimethyl derivative above, gave pale yellow prisms from methanol, m. p. 88—90°, of 1-(2-*nitrophenyl*)*naphthalene* (Found: C, 77·0; H, 4·5; N, 5·8. $C_{16}H_{11}O_2N$ requires C, 77·1; H, 4·4; N, 5·6%). Reduction of this nitro-compound (1·20 g.) by palladium–strontium carbonate (0·5 g.) in acetone (10 ml.) for 6—7 hours followed by crystallisation of the product from light petroleum (b. p. 40—60°) gave, slowly, cream crystals of 1-(2-*aminophenyl*)*naphthalene*, softening at 61°, melting at 65° (0·74 g.; yield, 70%) (Found: C, 87·8; H, 6·0; N, 6·5. $C_{16}H_{13}N$ requires C, 87·7; H, 5·9; N, 6·4%). Reduction with stannous chloride in glacial acetic acid saturated with hydrogen chloride gave the amine in poorer yield (30%). Diazotisation of this amine as for the corresponding 2 : 4-dimethyl derivative but in acetic acid solution gave leaflets of fluoranthene (yield, ~ 48%) from methanol, but more concentrated solutions gave prisms which, alone or mixed with genuine fluoranthene, had m. p. 110°. The picrate, orange prisms from ethanol, had m. p. 182—183° alone or mixed with fluoranthene picrate.

2 : 3 : 4-*Trimethylfluoranthene* (IV)—(II) (0·62 g.) and phosphoric oxide (1·24 g.) were heated at 250° for 1½ hours. After treatment with water, extraction with glacial acetic acid gave a brown insoluble residue (0·24 g.) and a solution which deposited a dirty yellow solid, thereafter insoluble. The acetic acid filtrate mixed with excess of picric acid solution in acetic acid gave a scarlet precipitate which when treated with dilute ammonia solution and washed gave a canary-yellow solid. After crystallisation from glacial acetic acid it was necessary in order to obtain an analytically pure specimen to pass the benzene solution through a column of alumina and crystallise the recovered material from acetone and then from ethanol. The pale green crystals of 2 : 3 : 4-*trimethylfluoranthene* (IV) so obtained softened at

131° and melted at 133—134°, as did a mixture with the substance previously described but not identified (France, Maitland, and Tucker, *loc. cit.*, p. 1744) (Found: C, 93·4; H, 6·7. $C_{15}H_{16}$ requires C, 93·4; H, 6·6%). Micro-hydrogenation (palladium black–acetic acid) indicated the presence of 2·07 double bonds. With picric acid in ethanol (IV) gave needles of a scarlet *picrate*, m. p. 208° (Found: C, 73·6; H, 4·9; N, 5·9. $2C_{15}H_{16},C_6H_3O_7N_3$ requires C, 73·6; H, 4·9; N, 5·9%). (IV) with *s*-trinitrobenzene in ethanol gave fine yellow needles of the *complex*, m. p. 228—230° (Found: C, 65·7; H, 4·4; N, 9·2. $C_{15}H_{16},C_6H_3O_6N_3$ requires C, 65·6; H, 4·2; N, 9·2%).

Oxidation of (III).—(III) (0·57 g.), sodium dichromate (6 g.), and glacial acetic acid (25 ml.) were boiled for 9 hours and the mixture worked up as previously described (France, Tucker, and Forrest, *loc. cit.*, p. 10), giving 1-acetylfluorenone (0·02 g.) and fluorenone-1-carboxylic acid (0·16 g.; yield, 29%).

Oxidation of (IV).—(IV) (0·49 g.), sodium dichromate (6 g.), and glacial acetic acid (25 ml.) were boiled for 18 hours. Working as above, 1-acetylfluorenone (0·01 g.) and fluorenone-1-carboxylic acid (0·24 g.; yield, 54%) were isolated. In each of the above experiments the identity of the 1-acetylfluorenone was established by m. p. of a mixture with previously analysed material, and by conversion, by means of iodine and dilute potassium hydroxide solution in methanol, into iodoform and the salmon-pink fluorenone-1-carboxylic acid.

Fluorene-1-carboxylic Acid.—Fluorenone-1-carboxylic acid (5 g.), lightly amalgamated granulated zinc (12 g.), and a mixture of glacial acetic acid and concentrated hydrochloric acid (1 : 1 by vol.; 150 ml.) were boiled vigorously for 1 hour. The colourless solution was poured into dilute acid and the precipitated solid crystallised from glacial acetic acid to give fluorene-1-carboxylic acid, m. p. 245° (4·5 g.; yield, 95%) (cf. Fieser and Seligman, *loc. cit.*). The *amide*, prepared in the usual way, crystallised from glacial acetic acid in laminae softening at 240°, melting at 249° (Found: C, 80·4; H, 5·4; N, 6·7. $C_{14}H_{11}ON$ requires C, 80·4; H, 5·3; N, 6·7%).

9-Fluorenol-1-carboxylic Acid.—(*a*) Fluorenone-1-carboxylic acid (16·8 g.) was reduced by magnesium (10 g.) in lime-dried, boiling methanol (300 ml.). The reaction, which was apt to be vigorous, gave a green then pale brown solution. It was poured into 25% hydrochloric acid. The precipitated alcohol-acid (16·4 g.; yield, 97%) was pure enough for subsequent use, but much was lost in complete purification by dissolving it in methanol, concentrating the solution, and adding 3 times the volume of hot glacial acetic acid to give *9-fluorenol-1-carboxylic acid* (8·05 g.; yield, 48%). The acid crystallised from toluene, then softened at 184°, melting at 196° (Found: C, 74·5; H, 4·5. $C_{14}H_{10}O_3$ requires C, 74·3; H, 4·4%).

(*b*) Fluorenone-1-carboxylic acid was hydrogenated by means of hydrogen and Raney nickel (Pavlic and Adkins, *J. Amer. Chem. Soc.*, 1946, **68**, 1471) in a mixture of ethanol (previously left over Raney nickel) and 10% potassium hydroxide solution. Absorption was rapid. Again, nearly theoretical yields of the practically pure alcohol-acid were obtained, but again purification caused considerable loss.

Similarly, fluorenone (0·9 g.) by means of Raney nickel (~ 1 g.) in ethanol (purified by Raney nickel; 40 ml.) containing 10% potassium hydroxide solution (1 ml.) was reduced in 5 minutes to fluorenol (0·73 g.; yield, 80%) (cf. Delépine and Horeau, *Bull. Soc. chim.*, 1937, **4**, 43, who dispensed with alkali but worked at 96°). We find that reduction at room temperature is incomplete in 55 minutes in absence of alkali. Addition of platinic chloride had apparently no effect (cf. *idem*, *loc. cit.*; Smith and Lieber, *J. Amer. Chem. Soc.*, 1936, **58**, 1417; Reasenberg, Lieber, and Smith, *ibid.*, 1939, **61**, 384). Reduction of fluorenone by means of palladium on charcoal (5%) (Cheronis and Levin, *J. Chem. Educ.*, 1944, **21**, 606) was slow. Thus with the quantities given above, but with palladium–charcoal (0·2 g.) in place of Raney nickel, reduction took 4 hours, but the yield of (nearly pure) fluorenol was ~ 90% (cf. also Bachmann, *J. Amer. Chem. Soc.*, 1933, **55**, 773; Miller and Bachman, *ibid.*, 1935, **57**, 770; France, Maitland, and Tucker, *loc. cit.*, p. 1741).

9-Chlorofluorene-1-carboxyl Chloride.—9-Fluorenol-1-carboxylic acid (3·2 g.) in chloroform (30 ml.) was treated portionwise with phosphorus pentachloride (10 g.); the mixture, after being boiled for ½ hour, was cooled in ice, the separated crystalline product extracted with hot chloroform (in which 9-chlorofluorene-1-carboxylic acid is insoluble), and the solution left to crystallise. More crystals were obtained by subsequently treating the filtered solution with light petroleum (b. p. 60—80°). *9-Chlorofluorene-1-carboxyl chloride* was obtained in faintly greenish prisms, softening at 145°, and melting at 158° (2·43 g.; yield, 64%) (Found: C, 63·8; H, 3·0. $C_{14}H_8OCl_2$ requires C, 63·9; H, 3·0%). Treatment of this acid chloride with hot glacial acetic acid gave large prisms of *9-chlorofluorene-1-carboxylic acid* (100%) which softened at 190°, and melted with effervescence at 242° (Found: C, 68·5; H, 3·6. $C_{14}H_9O_2Cl$ requires C, 68·7; H, 3·7%).

Methyl 9-chlorofluorene-1-carboxylate, produced by boiling the above acid chloride with absolute methanol until a clear solution was obtained, separated, on cooling, in long needles, m. p. 89—92° (yield, 100%) (Found: 69·5; H, 4·1. $C_{15}H_{11}O_2Cl$ requires C, 69·7; H, 4·25%).

9-Bromofluorene-1-carboxylic Acid.—9-Fluorenol-1-carboxylic acid (8 g.) was dissolved in warm glacial acetic acid (45 ml.) and saturated with dry hydrogen bromide. The crystalline *bromo*-compound which separated crystallised from glacial acetic acid in faintly yellow stout prisms which decomposed slightly at 218° and were molten at 242° (8·7 g.; yield, 85%) (Found: C, 58·2; H, 3·3; Br, 27·5. $C_{14}H_9O_2Br$ requires C, 58·1; H, 3·1; Br, 27·7%).

9-Bromofluorene-1-carboxyl Chloride.—9-Bromofluorene-1-carboxylic acid (10 g.) in chloroform (60 ml.) was treated with phosphorus pentachloride (10 g.), the solution being then boiled until all the acid had dissolved and hydrogen chloride ceased to be evolved. The liquor, decanted from unchanged phosphorus pentachloride, was treated with light petroleum (b. p. 60—80°); the crystals of the *acid chloride* so obtained recrystallised from benzene in thick prismatic plates, m. p. 169—172° (8·3 g.; yield, 85%) (Found: C, 54·7; H, 2·7; Cl + Br, 37·7. $C_{14}H_8OClBr$ requires C, 54·6; H, 2·6; Cl + Br, 37·5%).

Methyl 9-bromofluorene-1-carboxylate, prepared as described for the corresponding chloro-compound, separated from methanol in long needles, m. p. 102—104° (1·9 g.; yield, 97%) (Found: C, 59·6; H, 3·8; Br, 26·5. $C_{15}H_{11}O_2Br$ requires C, 59·4; H, 3·6; Br, 26·4%).

9-Bromofluorene-1-carboxyamide, prepared in the usual way from the acid chloride and ammonia, crystallised from glacial acetic acid in felted needles, m. p. 238° (decomp.) (Found: C, 58·6; H, 3·7; N, 5·0; Br, 27·6. $C_{14}H_{10}ONBr$ requires C, 58·4; H, 3·5; N, 4·9; Br, 27·8%).

9-Iodofluorene-1-carboxylic acid was prepared by the action of sodium iodide on 9-bromofluorene-1-carboxylic acid in acetone solution. It crystallised from acetone in yellow needles, m. p. 175° (decomp.) (yield, 80%) (Found: I, 37·6. $C_{14}H_9O_2I$ requires I, 37·7%).

Ethyl 9-Chlorofluorene-1-carbonylacetoacetate.—9-Chlorofluorene-1-carboxyl chloride (0·66 g.) was added solid to a gelatinous suspension of ethyl sodioacetoacetate [from sodium (0·06 g.) and ethyl acetoacetate (0·33 g.)] in ether (10 ml.). After thorough shaking the mixture was boiled for 2 hours. Very dilute acetic acid was added to render the solution acid, the ethereal layer was thoroughly washed to remove acid, and after evaporation to dryness the residue was crystallised from glacial acetic acid. The *product* was extracted with light petroleum (b. p. 60—80°), filtered from an insoluble residue, and allowed to crystallise to give clusters of faintly cream-coloured prisms, softening at 130°, melting at 133—134° (0·3 g.; yield, 33%) (Found: C, 67·4; H, 4·8; Cl, 9·7. $C_{20}H_{17}O_4Cl$ requires C, 67·3; H, 4·8; Cl, 10·0%).

3-(9-Chlorofluorene-1-carbonyl)butan-2-one.—A suspension of 9-chlorofluorene-1-carboxyl chloride (0·26 g.) in ether (20 ml.) and ethyl sodiomethylacetoacetate [(from sodium (0·06 g.) and ethyl methylacetoacetate (0·36 g.)] was boiled for ½ hour. The whole was treated with very dilute acetic acid and the ether extract evaporated. The residual *compound* crystallised from light petroleum (b. p. 60—80°) in large colourless rhombohedra, m. p. 131—132°. The absence of colour and lowered m. p. of a mixture showed it to be different from the lower homologue above described (Found: C, 72·2; H, 5·3; Cl, 11·75. $C_{18}H_{15}O_2Cl$ requires C, 72·4; H, 5·0; Cl, 11·9%). A small amount of material insoluble in light petroleum was shown to be 9-chlorofluorene-1-carboxylic acid, m. p. 242°.

Ethyl 9-Bromofluorene-1-carbonylacetoacetate.—9-Bromofluorene-1-carboxyl chloride (6 g.) was added to ethyl sodioacetoacetate (6·1 g., *i.e.*, excess) suspended in ether (100 ml.) and the mixture boiled for 3 hours. The *product* obtained in the usual way crystallised from methanol in cream-coloured prisms, m. p. 128—130° (Found: C, 59·7; H, 4·2; Br, 20·1. $C_{20}H_{17}O_4Br$ requires C, 59·9; H, 4·2; Br, 19·9%).

Ethyl 9-iodofluorene-1-carbonylacetoacetate was prepared from the above bromo-compound by treatment with sodium iodide in acetone; it crystallised from methanol in yellow prisms, m. p. 115—120°. Owing to its instability, analytical results were valueless.

Oxidation of Fluoranthene to Fluorenone-1-carboxylic Acid.—A solution of chromic acid (7 g., *i.e.*, 30% excess) in water (5 ml.) and glacial acetic acid (5 ml.) was added during 10 minutes to a boiling solution of fluoranthene (2·02 g.) in glacial acetic acid (25 ml.), the residual oxidising mixture being rinsed in with more (5 ml.) glacial acetic acid. After being boiled for a further hour the hot mixture was poured into dilute sulphuric acid (1 : 4 v/v, 200 ml.). After cooling, the precipitated solid was collected, washed with dilute acid and water, dissolved in ammonia solution, the solution filtered from a very small amount of insoluble material, and the acid reprecipitated with sulphuric acid solution. It was nearly pure; m. p. 185° (yield, 73%). Purification by crystallisation from glacial acetic acid resulted in a 5—10% loss. The use of an oxidising mixture consisting of sodium dichromate (12 g., *i.e.*, 50% excess), water (7 ml.), glacial acetic acid (10 ml.), and concentrated sulphuric acid (2 ml.) gave slightly higher yields of the acid, but owing to separation of inorganic salts the solutions bumped badly, and accordingly this modification is not recommended.

Ethyl fluorenone-1-carboxylate was prepared from the acid, ethanol, and hydrogen chloride; it crystallised from methanol in greenish-yellow rods, m. p. 85—86° (Found: C, 76·3; H, 5·0. $C_{16}H_{12}O_3$ requires C, 76·2; H, 4·8%). It gave a *semicarbazone* which crystallised from ethyl acetate as a mixture of feathery and prismatic crystals which softened at 180° and melted at 202° (Found: C, 65·8; H, 4·9. $C_{17}H_{15}O_3N_3$ requires C, 66·0; H, 4·9%). A 2 : 4-*dinitrophenylhydrazone* crystallised from glacial acetic acid in salmon-red leaflets, softening at 248° and melting at 253° (Found: C, 61·1; H, 3·9; N, 13·0. $C_{22}H_{14}O_6N_4$ requires C, 61·1; H, 3·7; N, 13·0%).

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UNIVERSITY OF GLASGOW. [*Received, September 1st*, 1947.]

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227. *The Constitution of Mesquite Gum. Part I. Isolation of 6- and 4-Glucuronosidogalactose.*

By J. I. CUNNEEN and F. SMITH.

Mesquite gum, the neutral salt of an acidic polysaccharide, gives on hydrolysis with dilute mineral acid a mixture of L-arabinose and a degraded mesquitic acid. Hydrolytic cleavage of the latter yields a mixture of two aldobiuronic acids (I) and (II) together with other reducing fragments (galactose and glucuronic acid) of the complex molecule.

Studies of the methylation of the aldobiuronic acids [(I) and (II)] and subsequent hydrolysis revealed their structures. Methylation affords a mixture of the methyl derivatives of 4-glucuronosidogalactose (III) and 6-glucuronosidogalactose (IV). One of these (IV) is convertible directly into the characteristic crystalline amide (V). Methanolysis of the mixture of the methyl derivatives of the two aldobiuronic acids gives almost equal amounts of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (VI) and a mixture of 2 : 3 : 6- and 2 : 3 : 4-trimethyl methylgalactosides, (XI) and (XIV), respectively. These three products were identified by conversion into characteristic crystalline derivatives, the first being trans-

formed into the amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside and into 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X); the second into 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII); and the third into the phenylhydrazide of 2 : 3 : 4-trimethyl galactonic acid (XVII).

THE examination of mesquite gum, the first part of which is reported here, was carried out in 1939 (Cunneen, Ph.D. Thesis, Birmingham, 1940) and was a part of an investigation into the constitution of plant gums on which one of us (F. S.) has been engaged for some years.

Gum mesquite exudes from the stem and branches of the mesquite tree, *Prosopis juliflora*, DC, which grows in the semi-arid regions of the South-Western States of the U.S.A., in Argentina, and in Chile. The gum is readily soluble in water and has been shown by Anderson and Otis (*J. Amer. Chem. Soc.*, 1926, **48**, 3172) to be the neutral salt of a complex acidic polysaccharide like damson gum (Hirst and Jones, *J.*, 1939, 1482), cherry gum (Jones, *J.*, 1939, 558), gum arabic (Neubauer, *J. pr. Chem.*, 1854, **62**, 193; Smith, *J.*, 1939, 744), and other plant gums.

Anderson and Otis (*loc. cit.*) also established the fact that mesquite gum contained L-arabinose and D-galactose since these sugars were isolated in the crystalline condition by hydrolysis of the gum with acid. This work also led to the suggestion that a third constituent of the polysaccharide was a monomethyl derivative of D-glucuronic acid (Anderson and Otis, *J. Amer. Chem. Soc.*, 1930, 4461). We have confirmed these observations but as yet there is no proof of the location of the methyl group in the glucuronic acid residue.

Our attention was first directed to a study of the hydrolysis products of this gum, and we have been able to isolate two aldobiuronic acids, 4-glucuronosidogalactose (I) and 6-glucuronosidogalactose (II), the constitutions of which have been proved by the isolation and characterisation of crystalline derivatives.

The gum, or the mesquitic acid obtained by pouring an aqueous acidified solution of the gum into alcohol, undergoes ready hydrolysis when heated with 0·01N-sulphuric acid with formation of L-arabinose and a degraded mesquitic acid. The latter does not appear to contain arabinose and is composed of galactose and glucuronic acid residues only. In this respect degraded mesquitic acid is analogous to degraded arabic acid, which has already been shown to contain galactose and glucuronic acid (Smith, *J.*, 1939, 1724). Further work has been done on the degraded mesquite acid and reference to it will be made in a later communication.

Prolonged hydrolysis of mesquitic acid or degraded mesquitic acid with dilute sulphuric acid (3%) afforded a mixture containing two aldobiuronic acids and reducing fragments. The latter probably consist of galactose and glucuronic acid. The barium salts of the glucuronic and aldobiuronic acids were readily separated from the reducing sugars by precipitation with alcohol.

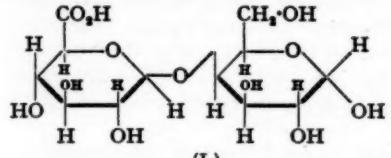
Methylation of the barium salts of the acids, first with methyl sulphate and sodium hydroxide, and then with silver oxide and methyl iodide, gave a product from which a mixture of the two methylated aldobiuronic acids (III) and (IV) was separated.

This mixture could not be separated by fractional distillation but, on treatment of it with methyl-alcoholic ammonia, one of its constituents (IV) gave the crystalline amide (V) of hexamethyl 6-β-D-glucuronosido-β-methyl-D-galactopyranoside, obtained previously from gum arabic (Jackson and Smith, *J.*, 1940, 74). This proved that one of the constituents had the constitution (IV), from which it follows that (II) is the aldobiuronic acid from which (IV) is derived.

Confirmation of the structure (II) assigned to one aldobiuronic acid as a result of the isolation of the crystalline amide and proof of the structure (I) of the second one was established as a result of the following observations. Methanolysis (*i.e.*, simultaneous hydrolysis and methyl-glycoside formation) of the mixture of the two methyl esters of the methylated aldobiuronic acids [(III) and (IV)] with methyl-alcoholic hydrogen chloride (8%) gave the methyl ester of the trimethyl methylglucuronoside (mixture of α- and β-isomers) (VI) and an almost equal amount of a mixture of the two trimethyl methylgalactosides (XI) and (XIV).

The methyl ester of the trimethyl methylglucuronoside (VI) was shown to be the 2 : 3 : 4-trimethyl derivative because upon treatment with methyl-alcoholic ammonia the α-isomer gave the crystalline amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside, identified by comparison with an authentic specimen (Smith, *J.*, 1939, 1732). The structure (VI) assigned to the methylated uronic acid was confirmed as follows. Hydrolysis of (VI) with N-sulphuric acid gave a reducing acid (VIII) which on oxidation with bromine furnished in solution the lactone (IX). Treatment of (IX) with 1% methyl-alcoholic hydrogen chloride effected esterification, and, on distillation in high vacuum, there was obtained the crystalline 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X). This lactone-ester was identical with an authentic specimen

prepared previously (Charlton, Haworth, and Herbert, *J.*, 1931, 2855; Robertson and Waters, *J.*, 1931, 1709; Smith, *J.*, 1939, 1733).



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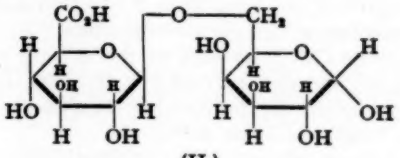

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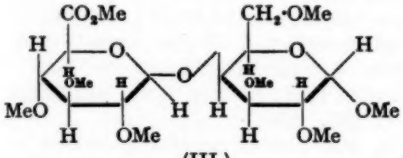

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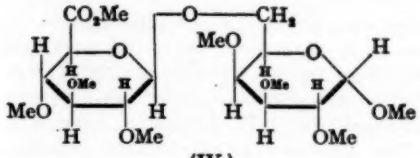

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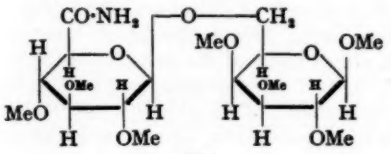

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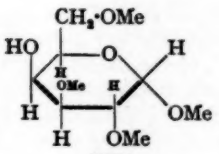

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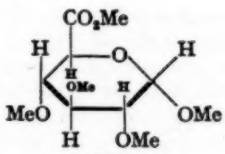

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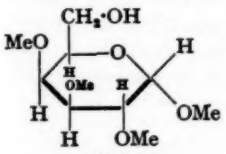

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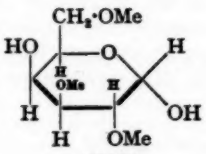

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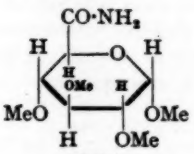

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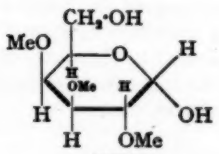

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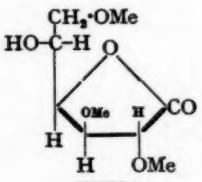

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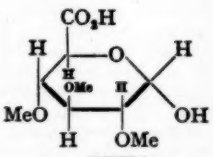

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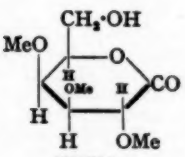

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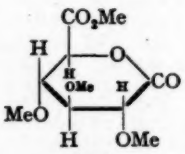

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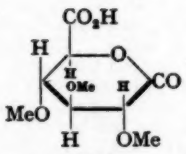

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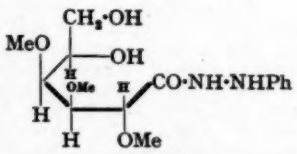

(XVII.)

The constitution of the two trimethyl methylgalactosides (XI) and (XIV) obtained by hydrolysis of the methylated aldobiuronic acids (III) and (IV) is based upon the following facts. Hydrolysis of the mixture of the two methylated galactose derivatives with 0·5N-sulphuric acid afforded a mixture of the two sugars (XII) and (XV) which gave rise on oxidation with bromine to the corresponding lactones (XIII) and (XVI) from which 2 : 3 : 6-trimethyl galactono-γ-lactone readily crystallised on nucleation. Purification of the crystalline trimethyl lactone gave a substance identical with an authentic specimen of 2 : 3 : 6-trimethyl galactono-γ-lactone (Haworth, Hirst, and Stacey, *J.*, 1932, 2481).

As much as possible of the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone was separated from the mixture containing the second trimethyl galactonolactone by extraction with ether–light petroleum. Indications were obtained that the unknown trimethyl lactone was a δ-lactone, although it was contaminated with 2 : 3 : 6-trimethyl galactono-γ-lactone, by reason of the fact

that an aqueous solution of the syrupy lactone displayed relatively rapid mutarotation in aqueous solution in a negative direction, reaching an equilibrium value in 9 hours. For comparison, the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII) showed relatively slow mutarotation in a positive direction ($[\alpha]_D$ — 44° changing to — 29° in 14 days). The trimethyl δ-lactone (XVI) present in the syrupy material proved to be 2 : 3 : 4-trimethyl galactono-δ-lactone, for on treatment with phenylhydrazine there was obtained the easily accessible characteristic crystalline phenylhydrazide (XVII) of 2 : 3 : 4-trimethyl galactonic acid (McCreath and Smith, *J.*, 1939, 387).

The presence of 2 : 3 : 4-trimethyl galactose can only be explained by the existence of an aldobiuronic acid in which the uronic acid moiety, in this case glucuronic acid, is joined through its reducing group to C_6 of a galactose unit, *i.e.*, the biose link is of the 1 : 6 type as shown in (II). This is in agreement with the previously established observation of the formation of the crystalline amide (V) of hexamethyl 6-β-D-glucuronosido-β-D-methylgalactopyranoside. This aldobiuronic acid containing the 1 : 6 link is identical with that obtained from gum arabic by prolonged hydrolysis (Haworth, Hirst, and Challinor, *J.*, 1931, 258).

The structure of the second aldobiuronic acid (I), also consisting of a galactose and a glucuronic acid unit, follows from the isolation and identification of the crystalline 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII). This lactone is derivable from either a 2 : 3 : 6-trimethyl galactofuranose or a 2 : 3 : 6-trimethyl galactopyranose unit. Hence the biose link in the aldobiuronic acid may join C_1 of the uronic acid residue either to C_4 of the galactose unit giving a structure for the aldobiuronic acid represented by (I), or to C_5 of the galactose unit in which case the aldobionic acid would be a glucuronosidogalactofuranose. If the aldobiuronic acid was a glucuronosidogalactofuranose, the linking of such an aldobiuronic acid to the rest of the molecule in the mesquite acid would be expected to be cleaved relatively easily by acid hydrolysis. The liberation of the aldobionic acid was found, however, to require rather prolonged hydrolysis. It is therefore designated 4-glucopyruronosidogalactopyranose and assigned the structure (I).

As far as the authors are aware, this is the first occasion on which the isolation of two aldobionic acids from a plant gum has been recorded. Its significance will be discussed in a later communication.

Experimental.

Preparation of Mesquitic Acid.—Mesquite gum (500 g.), dissolved in cold water (2 l.), was acidified with dilute hydrochloric acid (Congo-red), and the mixture poured slowly with stirring in a thin stream into ethyl alcohol (6 l.). The precipitated solid was washed twice by decantation with fresh alcohol, filtered, and pressed to remove as much alcohol as possible. After a repetition of this process, followed by precipitation of the mesquitic acid by pouring an aqueous solution of it into alcohol to remove mineral acid, the product was filtered off, washed with alcohol and then with ether, and dried in a vacuum. The resulting mesquitic acid was a fine white amorphous powder. It was free of inorganic salts, contained no mineral acid, and showed $[\alpha]_D^{18°}$ + 60° in water (*c*, 1·0) (Found: OMe, 2·9%; equiv., by titration with 0·02N-sodium hydroxide using phenolphthalein as indicator, 1350; iodine number, 2·6).

Hydrolysis of Mesquitic Acid and Formation of the Aldobionic Acids (I) *and* (II).—To a solution of mesquitic acid (300 g.) in water (1800 c.c.) was added concentrated sulphuric acid (36 c.c.) in water (120 c.c.). The solution was heated for 20 hours at 85°, decolourised with charcoal, treated with a slight excess of barium hydroxide (phenolphthalein), neutralised with carbon dioxide, and filtered. The precipitate was washed several times with hot water. The combined filtrate and washings were concentrated to a small volume (700 c.c.) under reduced pressure. When this solution was poured into methyl alcohol (6 l.) a mixture of the barium salts of uronic acids was precipitated. After being washed with alcohol and ether and dried in a vacuum at 40° it was obtained as a white amorphous powder (54 g.) (Found: OMe, 5·82; equiv., 495. Calc. for the barium salt of a mixture of aldobionic acids having one methoxyl group: OMe, 7·1%; equiv., 436). Further hydrolysis was effected by heating the barium salts (50 g.) for 14 hours at 85° with 5% sulphuric acid (360 c.c.). By working up the reaction mixture in the manner used above, the barium salts (20 g.) were isolated as a white powder (Found: OMe, 7·3%; equiv., 426).

The Methyl Esters of Hexamethyl 4- *and* 6-*Glucuronosidomethylgalactoside*, (III) *and* (IV).—A portion of the barium salts (13 g.) was dissolved in water (60 c.c.), and acetone (40 c.c.) was added until a slight turbidity was produced. This solution was then treated with methyl sulphate (200 c.c.) and sodium hydroxide (560 c.c. of 30% solution) at room temperature, the reagents being added gradually during 4 hours with vigorous stirring. After 6 hours' stirring the mixture was non-reducing to Fehling's solution; the temperature was then raised to 50°, and methyl sulphate (50 c.c.) and sodium hydroxide (140 c.c. of 30% solution) added in ½ hour after which time stirring was continued for a further 4 hours. The solution was cooled in an ice-bath, acidified with dilute sulphuric acid, and treated with ethyl alcohol (500 c.c.). The precipitated sodium sulphate was removed and washed with aqueous alcohol (60% ethyl alcohol). The combined filtrate and washings (2 l.) were made alkaline, concentrated almost to dryness under reduced pressure, and the partially methylated compound methylated further at 50° with methyl sulphate (250 c.c.) and sodium hydroxide (700 c.c. of 30% solution). The solution was acidified, the sodium sulphate was precipitated as before, and the solution, after being made just alkaline with dilute sodium hydroxide, was concentrated to about 100 c.c. A third methylation was then applied

using the method described for the second methylation. The resulting solution was concentrated to 700 c.c., acidified, and extracted 5 times with chloroform. The combined chloroform extracts, after being washed with water and dried ($MgSO_4$), gave on concentration a syrup (8·4 g.) consisting of partially methylated aldobionic acids.

A solution of the syrup (8·4 g.) in methyl iodide (25 c.c.) was boiled for 6 hours, and during this time dry silver oxide (10 g.) was gradually added. The residue, after removal of the methyl iodide by distillation, was extracted 6 times with hot acetone. Concentration of these extracts gave a syrup which was methylated twice in the same way. The syrup (7·4 g.), obtained by acetone extraction, furnished on distillation : *fraction* (*i*) (0·86 g.), b. p. 140—200° (bath temp.)/0·03 mm., $n_D^{18°}$ 1·4505; *fraction* (*ii*), a mixture of the methylated aldobionic acids (III) and (IV) (4·0 g.), b. p. 220—230° (bath temp.) /0·015 mm., $n_D^{18°}$ 1·4705, $[\alpha]_D^{20°}$ + 28° in water (*c*, 1·5) (Found : OMe, 50·0; equiv., 468. Calc. for $C_{20}H_{36}O_{12}$: OMe, 52·8%; equiv., 468). The undistilled residue amounted to 2·54 g. Since the mixture of aldobionic acids, fraction (ii) (3·9 g.), had a low methoxyl content, it was re-treated with silver oxide and methyl iodide and the product isolated in the usual way. There was no increase in the methoxyl content and hence the methylation was considered to be complete.

Examination of the Fully Methylated Aldobionic Acids (III) *and* (IV). *Identification of the Amide of Hexamethyl* 6-β-D-*Glucuronosido-β-methyl-*D-*galactopyranoside* (V).—Treatment of the fully methylated aldobionic acid (0·3 g.) with methyl-alcoholic ammonia at 0° for 38 hours followed by evaporation of the solvent gave the amide of hexamethyl-6-β-D-glucuronosido-β-methyl-D-galactopyranose (V) which crystallised from acetone–ether in colourless needles and had m. p. 195°, $[\alpha]_D^{16°}$ − 22° in water (*c*, 0·83) (Found : OMe, 46·3. Calc. for $C_{19}H_{35}O_{11}N$: OMe, 47·9%). A mixed m. p. with an authentic specimen, which had $[\alpha]_D^{20°}$ − 18° in water (*c*, 3·7) and m. p. 196°, showed no depression.

Hydrolysis of the Mixture of Methylated Aldobiuronic Acids (III) *and* (IV).—*Experiment I.* When a solution of a portion (1·27 g.) of fraction (ii) in 3% methyl-alcoholic hydrogen chloride (100 c.c.) was boiled under reflux the following changes in rotation were observed : + 21° (initial value); + 33° (3 hours); + 37° (5 hours); + 39° (7 hours); +43° (9 hours); + 47° (13 hours); + 48° (19 hours). Neutralisation of the acid with silver carbonate, followed by filtration and removal of the solvent, gave a syrupy residue which on distillation afforded : *fraction* (a) (0·47 g.), b. p. 100—118° (bath temp.)/0·01 mm., $n_D^{18°}$ 1·4508—1·4575 (Found : equiv., 390); *fraction* (b) (0·6 g.), b. p. 180—200° (bath temp.) /0·01 mm., $n_D^{18°}$ 1·4680 (Found : OMe, 50·0%; equiv., 450). The undistilled methylated aldobiuronic acid amounted to 0·2 g. It was apparent that hydrolysis was incomplete and that the mixture of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside and trimethyl methylgalactoside [fraction (*a*)] could not be separated readily by distillation.

Experiment II. When a solution of the mixture of methylated aldobiuronic acid [1·86 g. of fraction (ii)] in 8% methyl-alcoholic hydrogen chloride (100 c.c.) was boiled the rotational changes were as follows : + 23° (initial value); + 63° (1 hour); + 78° (2 hours); + 81° (3 hours); + 88° (5 hours); + 89° (7 hours); + 89° (9 hours). Isolation of the product as in Experiment I gave a mobile liquid (1·83 g.) most of which had b. p. < 160° (bath temp.) /0·01 mm. It was thus evident that hydrolysis could be effected by boiling 8% methyl-alcoholic hydrogen chloride.

The remaining mixture of the methylated aldobionic acids [fraction (ii)] was combined with the products obtained from Experiment I, including the undistilled residue (0·2 g.), and boiled for 10 hours with 100 c.c. of 8% methyl-alcoholic hydrogen chloride. The hydrolytic products were isolated as before and the combined cleavage fragments (3·5 g.) from this experiment and Experiment II were heated for 2 hours at 60° with barium hydroxide (1·5 g.) in water (100 c.c.). The excess of barium hydroxide was neutralised with carbon dioxide. The solution was filtered and evaporated to dryness under reduced pressure giving a syrup consisting of a mixture of the methylated galactosides (XI) and (XIV) and the barium salt of the trimethyl methylglucuronoside. Exhaustive ethereal extraction gave the mixture of galactosides (XI) and (XIV) as a colourless syrup (1·1 g.). The residual barium salt was left as a pale yellow solid (2·5 g.).

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (VI).—The yellow solid consisting of the barium salt of 2 : 3 : 4-trimethyl methylglucoronoside was converted into the methyl ester by boiling it for 6 hours with 2% methyl-alcoholic hydrogen chloride (100 c.c.). After separation of the precipitated barium chloride from the cooled solution by filtration, the solution was neutralised with silver carbonate and filtered, and the residue was washed well with hot methyl alcohol. Evaporation of the combined filtrate and washings gave a dark brown syrup which was dissolved in acetone. Addition of ether to the solution precipitated some inorganic impurity which was removed. Evaporation of the filtrate gave a clear yellow syrup which on distillation gave the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (VI) (1·0 g.), b. p. 135—155° (bath temp.) /0·02 mm., $n_D^{17°}$ 1·4485, $[\alpha]_D^{15°}$ + 80° in water (*c*, 1·4) (Found : OMe, 57·8; equiv., 263. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264). A residue of unhydrolysed methylated aldobionic acids remained (0·76 g.).

The Amide of 2 : 3 : 4-*Trimethyl α-Methylglucuronoside* (VII).—Treatment of the methylated glucuronic ester (VI) (0·1 g.) with methyl-alcoholic ammonia for 2 days at 0° followed by removal of the solvent under reduced pressure gave the crystalline amide (VII) of 2 : 3 : 4-trimethyl α-methylglucuronoside. Recrystallisation from ethyl alcohol–ether–light petroleum yielded colourless needles, m. p. and mixed m. p. 185°, $[\alpha]_D^{17°}$ + 137° in water (*c*, 0·7) (Found : C, 48·5; H, 7·55; N, 5·6; OMe, 48·7. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·75; N, 5·6; OMe, 49·8%).

2 : 3 : 4-*Trimethyl Glucosaccharo*-1 : 5-*lactone* 6-*Methyl Ester* (X).—A portion (0·76 g.) of the ester (VI) was heated on the boiling water-bath with N-sulphuric acid (30 c.c.) for 22 hours during which rotation changed from + 57° to + 43°. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure. Extraction of the resulting solids with methyl alcohol, followed by concentration of the extracts, gave a pale yellow solid (0·76 g.) which reduced Fehling's solution and gave a positive test for barium.

To the yellow solid (0·76 g.) in water (5 c.c.), bromine (1 c.c.) was added and the mixture left (4 days) at room temperature until a portion of the solution, when freed from bromine, no longer reduced Fehling's

solution. The excess of bromine was then removed by aeration, and the solution neutralised with silver carbonate, filtered and the filtrate treated with hydrogen sulphide to remove silver as silver sulphide. The solution was immediately evaporated to dryness and extracted 3 times with hot methyl alcohol. Concentration of the extracts gave the barium salt of 2 : 3 : 4-trimethyl saccharolactone (0·55 g.) which was converted into the methyl ester by boiling it for 8 hours with 1% methyl-alcoholic hydrogen chloride (50 c.c.). After removal of the precipitated barium chloride from the cooled solution, the latter was neutralised with silver carbonate and filtered, the residue being well washed with hot methyl alcohol. Evaporation of the filtrate and washings gave a syrup (0·4 g.) which on distillation gave 2 : 3 : 4-trimethyl glucosaccharolactone methyl ester (X) (0·21 g.), b. p. 133° (bath temp.) /0·03 mm., $n_D^{19°}$ 1·4585; the colourless distillate crystallised spontaneously. After crystallisation from ethyl alcohol–ether the 2 : 3 : 4-trimethyl glucosaccharo-1 : 5-lactone 6-methyl ester (X) had m. p. and mixed m. p. 107°, $[\alpha]_D^{16°}$ + 88° in water (*c*, 0·9) (after 20 minutes), changing in 29 hours to + 35° (equilibrium value) (Found : C, 48·3; H, 6·3; OMe, 50·0. Calc. for $C_{10}H_{16}O_7$: C, 48·4; H, 6·5; OMe, 50·0%).

Examination of the Methylated Galactosides.—Identification of 2 : 3 : 6-*trimethyl methylgalactoside* (XI) *and* 2 : 3 : 4-*trimethyl methylgalactoside* (XIV). Slow distillation of the mixture of methylated galactosides gave a colourless liquid (0·7 g.), b. p. 125° (bath temp) /0·01 mm., $n_D^{18°}$ 1·4600, $[\alpha]_D^{16°}$ + 42° in water (*c*, 1·0). For 2 : 3 : 6-trimethyl methylgalactoside $n_D^{19°}$ 1·4550 has been recorded (Haworth, Raistrick, and Stacey, *Biochem. J.*, 1935, **29**, 2668), and 2 : 3 : 4-trimethyl methylgalactoside has $n_D^{19°}$ 1·4640 (McCreath and Smith, *J.*, 1939, 387; Smith, *J.*, 1939, 1726). It appeared that no separation of the galactosides had been effected by distillation (Found : OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%).

Hydrolysis of the methylated galactosides. When a solution of the distilled galactosides (0·49 g.) in 0·5N-sulphuric acid (13·5 c.c.) was heated on the boiling water-bath for 8 hours, the rotation changed from $[\alpha]_D^{16°}$ + 41° (initial value) to $[\alpha]_D^{16°}$ + 72° (constant value). The solution was neutralised with barium carbonate, filtered, evaporated to dryness under diminished pressure, and the residue extracted with ether. Concentration of the ethereal solution gave a mixture of the free sugars (XII) and (XV) (0·41 g.) (Found : OMe, 40·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%).

Oxidation with bromine. The mixture of trimethyl galactoses (0·4 g.) was dissolved in water (5 c.c.), bromine (0·4 c.c.) was added, and the solution left at room temperature for 2 days. After this time a portion of the mixture, when freed from bromine by aeration, was non-reducing to Fehling's solution. By working up in the usual manner there was obtained a mixture of lactones (0·37 g.) which furnished on distillation a syrup (0·22 g.), b. p. 165° (bath temp.) /0·012 mm., $n_D^{19°}$ 1·4660 (Found : OMe, 39·8. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). After being kept for a few days the distillate had undergone partial crystallisation.

Identification of 2 : 3 : 6-*Trimethyl Galactono-γ-lactone* (XIII).—The partially crystalline mixture of lactones was triturated with a little ether–light petroleum; this removed much of the syrup, and the crystals which remained undissolved were freed from the rest of the adhering syrup by means of a porous tile. After crystallisation from ether the 2 : 3 : 6-trimethyl galactono-γ-lactone (XIII) had m. p. and mixed m. p. 101°, $[\alpha]_D^{18°}$ — 44° (initial value in water; *c*, 0·9), changing to $[\alpha]_D^{18°}$ — 29° in 14 days (Found : OMe, 42·45. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). An authentic specimen of 2 : 3 : 6-trimethyl galactono-γ-lactone showed $[\alpha]_D$ — 40° (initial value) in water (*c*, 1·6) changing in 14 days to — 28° (equilibrium value).

Identification of 2 : 3 : 4-*Trimethyl Galactono-δ-lactone* (XVI).—The syrupy portion of the mixture of lactones which dissolved in ether–light petroleum was recovered by removal of the solvent. The syrup so obtained (0·1 g.) had $[\alpha]_D^{17°}$ + 35° in water (*c*, 1·0) changing to $[\alpha]_D^{19°}$ + 10° in 22 hours (Found : OMe, 39·8. Calc. for $C_9H_{16}O_6$: OMe, 42·3%). The lactone syrup (0·1 g.) was heated with phenylhydrazine (0·05 g.) in dry methyl alcohol (1 c.c.) for 15 minutes. The alcohol was then removed and the mixture heated for 3 hours at 95°. After cooling, trituration with ether gave crystalline 2 : 3 : 4-trimethyl galactonic acid phenylhydrazide (XVII) (0·06 g.), m. p. and mixed m. p. 176°, $[\alpha]_D^{17°}$ + 36° in ethyl alcohol (*c*, 0·8) (after crystallisation from ethyl alcohol–ether) (Found : C, 54·8; H, 7·15; N, 8·85; OMe, 27·4. Calc. for $C_{15}H_{24}O_6N_2$: C, 54·9; H, 7·4; N, 8·5; OMe, 28·4%).

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228. *The Constitution of Mesquite Gum. Part II. Methylated Mesquite Gum.*

By J. I. CUNNEEN and F. SMITH.

Methylation of mesquite gum or its acetate with methyl sulphate and sodium hydroxide affords the corresponding methylated mesquitic acid. By means of silver oxide and methyl iodide the latter is converted into the methyl ester. Methanolysis of the methyl ester of methylated mesquitic acid by methyl-alcoholic hydrogen chloride gives rise to 2 : 3 : 5-trimethyl methyl-L-arabofuranoside (I) (3 mols.); 3 : 5-*dimethyl methyl-L-arabofuranoside* (V) (6—7 mols.); 2 : 4-dimethyl methyl-D-galactopyranoside (IX) (4 mols.); a dimethyl methylhexoside (probably a galactoside) (1 mol.) and the methyl ester of 2 : 3 : 4-trimethyl methyl-D-glucuronoside (XI) (2 mols.). With the exception of the unknown dimethyl methylhexoside, these constituents of the methyl gum have been identified by their conversion into characteristic crystalline derivatives. The structural significance of the formation of the various methyl-glycosides is discussed.

In Part I (Cunneen and Smith, preceding paper) there was described the isolation and characterisation of two aldobiuronic acids, 6- and 4-glucuronosidogalactose, which form part of the complex molecular structure of mesquite gum. This paper is concerned with the determination of the mode of linking between these two aldobionic acids and the sugar components, galactose and arabinose, which constitute the rest of the complex structure.

The various methyl derivatives of galactose, arabinose, and glucuronic acid obtained by cleavage of methylated mesquite gum have been separated and identified, and an estimate made of the proportion of each of the substances present in the repeating unit of the complex molecule. Although there has been some hesitation in the past in drawing conclusions from the isolation of partly methylated glycosides formed by hydrolysis of methylated polysaccharides, it is now recognised that these partly methylated sugars are of structural significance (cf. Freudenberg, *Ber.*, 1940, **73**, 609), and, because of this recognition, it has been possible to make some progress in the determination of the constitution of highly complex polysaccharides such as damson gum (Hirst and Jones, *J.*, 1946, 506) and gum arabic (Smith, *J.*, 1940, 1035). The same significance is to be attached to the isolation of dimethyl glucose from the cleavage of methylated glycogen and methylated starch.

As the fractional precipitation of gums is often somewhat difficult, an investigation as to the homogeneity of mesquite gum was made on the methyl derivative and not on the gum itself. The methylated gum was obtained by the action of methyl sulphate and sodium hydroxide on either the gum or its acetyl derivative. The methylated mesquitic acid so prepared was esterified, and completely methylated by treatment with methyl iodide and silver oxide. Fractional precipitation of the methyl ester of methylated mesquitic acid from ethereal solution with light petroleum showed that the main part of the material was essentially homogeneous; it had an equivalent weight of 1550, $[\alpha]_D$ + 55° in chloroform, and a methoxyl content of 40%.

When boiled with 2% methyl-alcoholic hydrogen chloride, the methyl ester of methylated mesquitic acid undergoes simultaneous hydrolysis and glycoside formation with formation of a mixture of methylated sugars, methyl esters of methylated uronic, and methylated aldobionic acids. Treatment of the cleavage fragments with dilute barium hydroxide solution converted the esters of the acids into their barium salts. Extraction of a dry mixture of the barium salts and the methylated glycosides with ether readily removed the latter and left the barium salts as a pale yellow powder.

The mixture of methylated glycosides was separated by fractional distillation; this was simple compared with the separation of the methylglycosides from methylated arabic acid (Smith, *J.*, 1940, 1035), mainly owing to the larger difference in the boiling points of the constituents and to the fact that there was no tendency to form constant-boiling mixtures. The methylglycosides were identified as 2 : 3 : 5-trimethyl methyl-L-arabofuranoside, 3 : 5-*dimethyl methyl*-L-*arabofuranoside*, and 2 : 4-dimethyl methyl-D-galactopyranoside.

2 : 3 : 5-Trimethyl methylarabofuranoside (I) was characterised by the following facts. It was hydrolysed by dilute sulphuric acid to the trimethyl arabinose (II) which on oxidation with bromine in aqueous solution afforded the characteristic crystalline lactone (III) (Baker and Haworth, *J.*, 1925, 365). When allowed to react with methyl-alcoholic ammonia, the lactone (III) gave the characteristic crystalline amide (IV) of 2 : 3 : 5-trimethyl L-arabonic acid (Pryde, Humphreys, and Waters, *J.*, 1931, 1298; Haworth, Hirst, and Oliver, *J.*, 1934, 1922; Hirst and Jones, *J.*, 1939, 496; Smith, *J.*, 1940, 1035).

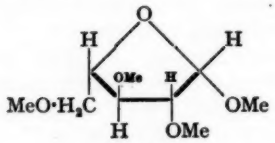
The structure assigned to the *dimethyl methylpentoside* (V) was ascertained in the following manner. Methylation of (V) with silver oxide and methyl iodide gave a trimethyl methylpentoside, identified as (I) by the reactions already outlined. This evidence demonstrated that the dimethyl methylpentoside was a derivative of methyl-L-arabofuranoside and that the two methyl groups must occupy the positions 2 : 3, 2 : 5, or 3 : 5.

To decide between these possibilities, the *dimethyl arabinose* (VI) obtained by hydrolysis of the arabinoside (V) was oxidised with bromine to the corresponding *dimethyl arabonolactone* (VII). This lactone was crystalline (m. p. 75°), and its slow rate of mutarotation in aqueous solution ($[\alpha]_D$ − 84° changing to − 69° in 28 days) indicated that it belonged to the γ-series of arabonolactones. The properties of the lactone (VII), and of the crystalline *amide* derived from it, distinguished it from the known 2 : 5-dimethyl L-arabonolactone (Smith, *J.*, 1939, 751) and also from the 2 : 3-isomer (Hirst and Jones, *J.*, 1938, 504; Smith, *J.*, 1939, 753).

It was thus inferred that the two methyl groups must be at C_3 and C_5, and this was confirmed by the fact that the amide (VIII) showed a positive test for α-hydroxy-amides (Weerman, *Rec. Trav. chim.*, 1917, **36**, 16).

Although at the time the lactone (VII) had not been described it has since been characterised

by White (*J. Amer. Chem. Soc.*, 1946, **68**, 272) who demonstrated the presence of a free hydroxyl group at C_2 by the fact that the dimethyl arabinose gives a phenylosazone without loss of methoxyl.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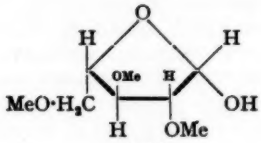
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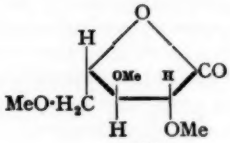
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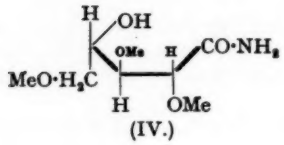
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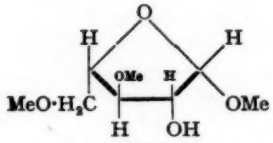
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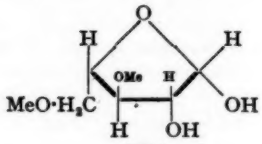
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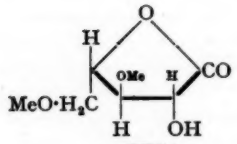
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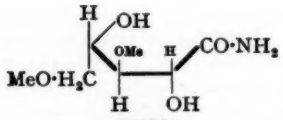
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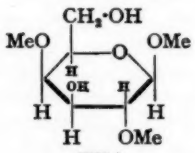
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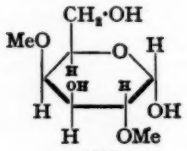
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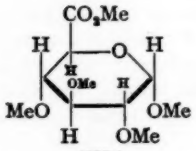
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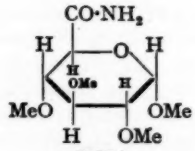
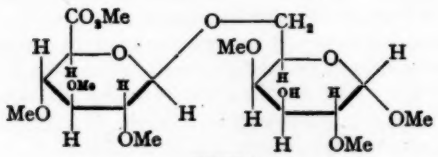

(XII.)

The third component of the mixture of methylated glycosides, the crystalline dimethyl methylhexoside (IX), was identified as 2 : 4-dimethyl β-methylgalactopyranoside by comparison with an authentic specimen previously obtained from arabic acid (Smith, *J.*, 1939, 1724). This constitution was confirmed by the observation that on hydrolysis with N-sulphuric acid (IX) gave 2 : 4-dimethyl galactose (X) as the crystalline monohydrate. Furthermore, treatment of (X) with ethyl-alcoholic aniline yielded the characteristic crystalline anilide (McCreath and Smith, *J.*, 1939, 387; Smith, *J.*, 1939, 1736).

The acidic components of the cleavage fragments isolated as barium salts were treated with acid methyl alcohol at room temperature, a procedure which esterifies, without cleaving the glycosidic bonds (Jackson and Smith, *J.*, 1940, 74). Fractional distillation of the mixture of ester glycosides thus formed gave the methyl ester of trimethyl methylglucuronoside (XI) and the methyl ester of a pentamethyl aldobiuronoside (XIII). A residue (F) remained.

The structure of the methyl ester of the trimethyl methylglucuronoside (XI) was proved by the observation that its treatment with methyl-alcoholic ammonia yielded the characteristic amide of trimethyl α-methylglucopyruronoside (XII), identified by comparison with an authentic specimen (Smith, *J.*, 1939, 1732).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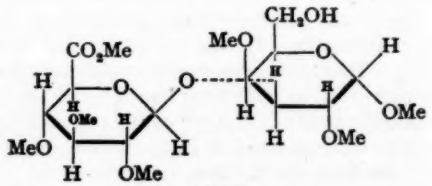

(XIV.)

The facts available strongly suggest that the methyl ester of the *pentamethyl methyl-aldobiuronoside* (XIII) is composed of a molecule of 2 : 3 : 4-trimethyl glucuronic acid joined

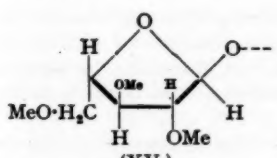
through its reducing group C_1 to position 6 of a molecule of 2 : 4-dimethyl methylgalactoside. On boiling the aldobionic ester (XIII) with 8% methyl-alcoholic hydrogen chloride, simultaneous hydrolysis and glycoside formation take place with production of almost equimolecular amounts of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and crystalline 2 : 4 dimethyl methylgalactopyranoside (IX). These were readily separable by fractional distillation and were identified in the manner already described.

The two fragments (XI) and (IX) of the methylated aldobiuronic acid would result from a biuronic acid composed of a 2 : 3 : 4-trimethyl methylglucuronic acid joined through its reducing group either to position 3 or to position 6 of the 2 : 4-dimethyl methylgalactopyranoside. The two possibilities are shown by the formulations (XIII) and (XIV) respectively both of which would give on cleavage the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and crystalline 2 : 4-dimethyl methylgalactopyranoside (IX) as required by the experimental facts. Previous investigations have established the fact that two aldobiuronic acids are produced by hydrolysis of mesquite gum, namely, 6- and 4-glucuronosido-galactose (Cunneen and Smith, *loc. cit.*). Only the former will allow of the formation of a 2 : 4-dimethyl galactose residue, and for this reason it is believed that in the methylated aldobionic acid obtained from methylated mesquitic acid there is present a 1 : 6 link and that it is therefore to be represented by (XIII) and not by (XIV).

The existence of an aldobiuronic acid in mesquite gum having a 1 : 3-glycosidic linkage cannot, however, be entirely dismissed on the evidence produced above because it may be that such an aldobionic acid would be more easily hydrolysed than those containing the 1 : 6 and the 1 : 4 linkages.

The incompletely hydrolysed acid fragments (residue F, see above) gave upon further hydrolysis with acid methyl alcohol more 2 : 3 : 4-trimethyl methylglucuronoside and 2 : 4-dimethyl methylgalactoside, but there still remained an acid-resistant residue approximating analytically to a methylated aldobionic acid. This required treatment with methyl-alcoholic hydrogen chloride in a sealed tube at 130° before it underwent cleavage to give a trimethyl methylglucuronoside and a dimethyl methylhexoside. The identity of the latter was not established, but it is believed that these two fragments are derived by hydrolysis of a methylated aldobiuronic acid which corresponds to the 4-glucuronosidogalactose previously isolated from mesquite gum (Cunneen and Smith, *loc. cit.*).

As a result of these constitutional studies it is at once apparent that although the structure of methylated mesquite gum, as a whole, is complicated, it is nevertheless characterised by some simple features. Thus the characterisation of the 2 : 3 : 5-trimethyl methyl-L-arabinoside (I) and the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) prove that these units must constitute " end " residues in the complex and consequently they are joined to other units in the methylated polysaccharide gum by glycosidic bonds shown by broken lines in formula (XV) and (XVI).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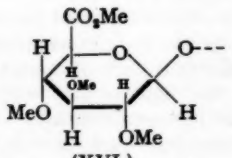
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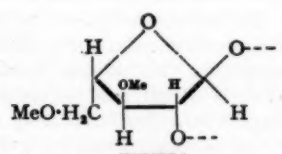
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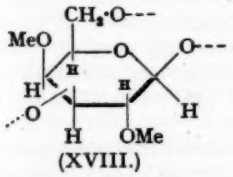
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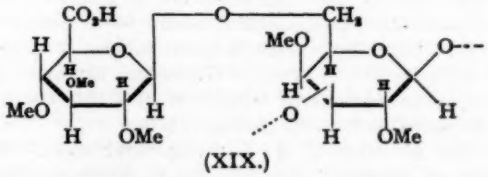
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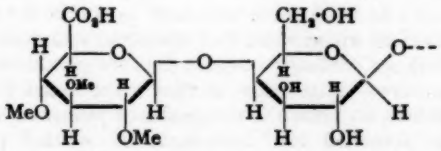

(XX.)

The dimethyl methyl-L-arabinoside with its substituent methyl groups in positions 3 and 5 can arise only from L-arabofuranose residues which are joined by glycosidic bonds to other units in the complex as in (XVII).

By the same reasoning it is clear that the 2 : 4-dimethyl methylgalactoside is produced from galactose units involved in glycosidic union with other units through positions 3 and 6 as in (XVIII) (see White, *loc. cit.*).

Isolation and characterisation of 6 β-D-glucuronosido-D-galactose by direct hydrolysis of mesquite gum establishes the fact that some of the end glucuronic acid units are joined to position 6 of a galactose residue (Cunneen and Smith, *loc. cit.*). This is now confirmed by the isolation of the methyl ester of a pentamethyl 6-glucuronosidomethylgalactoside from the methylated mesquitic acid. The experimental proof that this pentamethyl derivative is composed of a unit of 2 : 3 : 4-trimethyl glucuronic acid joined by a 1 : 6-linkage to a unit of 2 : 4-dimethyl galactose enables the further deduction to be made that the galactose residue in the aldobionic acid must be joined to other residues of the complex through positions 1 and 3 as in (XIX).

Since the existence of an aldobiuronic acid having a 1 : 4-linkage has been established by the isolation of 4-glucuronosidogalactose from the hydrolytic fragments of mesquite gum (Cunneen and Smith, *loc. cit.*) it is also apparent that some glucuronic acid units are joined to other galactose units through position 4 as in (XX). The manner in which the 4-glucuronosido-galactose fragment is linked to the rest of the mesquitic acid molecule has not yet been determined, but if it proves to be correct that the dimethyl hexose isolated as described above from methylated mesquitic acid is in fact derived from the 4-glucuronosidogalactose, then it is obvious that this aldobionic acid is attached to the rest of the complex by two linkages. One link involves the reducing group (C_1) of the galactose unit, the other engages one of the positions 2, 3, or 6. The elucidation of the structure of this dimethyl galactose unit will be the subject of further investigation.

Plant gums are composed of several kinds of sugar units, some of which may be present as pyranose and others as furanose forms. Most plant gums contain the oxidised form of a hexose such as glucuronic or galacturonic acid, and some contain the reduced form of a hexose such as rhamnose (gum arabic) or fucose (gum tragacanth), but, so far, such reduced forms of the sugars as hexitols or pentitols have not been detected. Another characteristic of plant gums is that the units are joined by several types of glycosidic linkages. The gum now being discussed is a typical plant gum inasmuch as the component monosaccharide residues consisting of galactose, arabinose, and glucuronic acid are mutually joined by no less than four types of linkage, namely the 1 : 2-pentose and the 1 : 3-, 1 : 4-, and 1 : 6-hexose types. The presence of the 1 : 2-linkage is deduced from the isolation of 3 : 5-dimethyl arabinose, the 1 : 3-linkage from the isolation of 2 : 4-dimethyl galactose, the 1 : 4-linkage from the characterisation of 4-glucuronosidogalactose, and the fourth type, the 1 : 6-linkage, follows from the isolation of 6-glucuronosidogalactose and 2 : 4-dimethyl galactose.

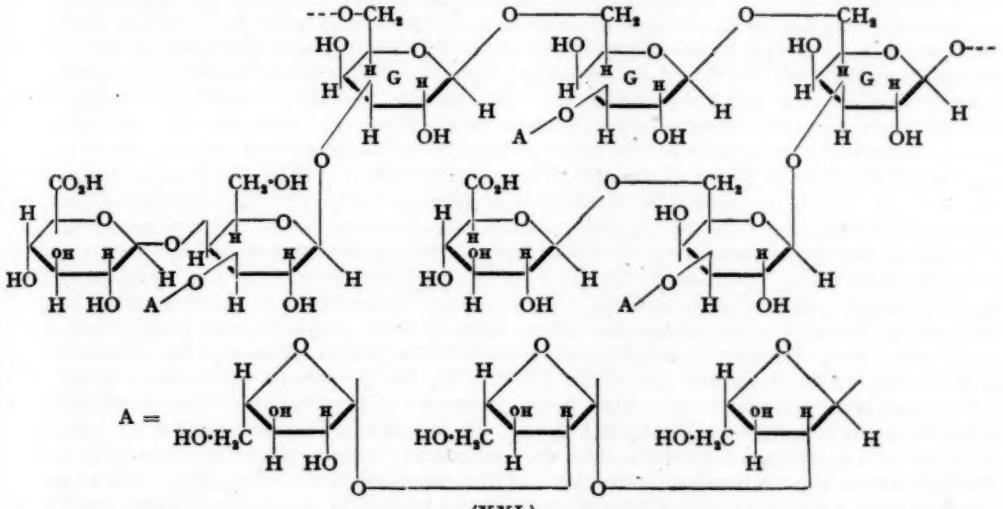
The identification of the products of hydrolysis of the methylated polysaccharides is not an easy problem, and some difficulty attends the determination of the amounts of each of these fragments. No unique solution of the structure of mesquite gum can, therefore, be advanced, but it is of interest to consider, in the light of the available evidence, the possible types of complex structure that may represent its constitution.

Calculations based upon the weights of the various constituents of the mixture of the cleavage fragments obtained from the methylated mesquite gum indicate that the approximate relative proportions of the fragments are as follows: 2 : 3 : 5-trimethyl methylarabinoside (3 mols.); 3 : 5-dimethyl methylarabinoside (6—7 mols.); 2 : 4-dimethyl methylgalactoside (4 mols.); 2 : 3 : 4-trimethyl methylglucuronoside (2 mols.); an unknown dimethyl methylhexoside (? galactoside) (1 mol.). Glycosidic union of these 17 fragments will give a repeating unit having an equivalent weight of 1250 for the mesquitic acid (found, 1350) and 1570 for the methylated substance (found, 1550).

The isolation of 2 : 3 : 5-trimethyl and 3 : 5-dimethyl arabinose shows that the pentose units are present in the complex as furanose units, while the identification of the galactose units as a 2 : 4-dimethyl derivative and the glucuronic acid as the 2 : 3 : 4-trimethyl derivative, and the very probable formation of an unidentified dimethyl galactose from the aldobionic acid which possesses the 1 : 4-linkage, strongly suggest that the galactose and glucuronic acid units all have the pyranose structure. It is believed that there exists in mesquitic acid a nucleus, relatively stable to acid hydrolysis, which is composed of pyranose units of glucuronic acid and galactose and to which is attached the arabofuranose residue (cf. arabic acid). Such a

representation derives support from the fact that hydrolysis of the gum with 0·1N-sulphuric acid gives arabinose and a degraded mesquitic acid which is composed of glucuronic acid and galactose residues only. The methylated gum is degraded in an analogous fashion by boiling 0·5% methyl-alcoholic hydrogen chloride. A mixture of 3 : 5-dimethyl and 2 : 3 : 5-trimethyl methylarabofuranoside is produced, while the stable nucleus of methylated degraded mesquitic acid, composed solely of galactose and glucuronic acid units, remains unaffected. The hydrolysis of this relatively stable methylated nucleus demands a much more drastic treatment than that required for the cleavage of the arabofuranoside links, an observation which supports the view that the residues of galactose and glucuronic acid, of which this nucleus is composed, are of the pyranose type.

The presence of branched chains is clearly proved not only by the isolation of 2 : 4-dimethyl galactose but by the isolation of three molecular proportions of 2 : 3 : 5-trimethyl arabinose and two of 2 : 3 : 4-trimethyl glucuronic acid. The arabinose units which afford the 2 : 3 : 5-trimethyl derivative and the glucuronic acid units from which the corresponding 2 : 3 : 4-trimethyl derivative arises, constitute five end groups in the structure of a repeating unit composed of approximately seventeen sugar residues. This high proportion of end groups can arise only from a multiple branched-chain structure.



(XXI.)

Several structures may be assigned to mesquitic acid on the basis of the above evidence. One possible formulation of the repeating unit is shown by (XXI); this consists of three galactose units G joined by 1 : 6-linkages to form a main chain, and to position 3 of any two of the three galactose units of this main chain are attached the two branching side chains consisting of two aldobiuronic acids the presence of which in mesquitic acid has been shown both in this work and in that recorded in Part I. Thus five galactose units and two glucuronic acid residues constitute the stable nucleus of degraded mesquitic acid.

To this stable nucleus are attached three other side chains designated by (A) in (XXI). These three groups are composed entirely of the arabofuranose units which are split off by mild acid hydrolysis from the relatively stable nucleus of mesquitic acid. Of the methylated arabofuranose units that are formed from the methyl ester of methylated mesquitic acid by methanolysis, three are fully methylated and must constitute terminal units. The remaining arabinose units appear as the 3 : 5-dimethyl derivatives and must therefore be distributed as non-terminal units of three chains in which the arabofuranose units are mutually joined by 1 : 2-glycosidic linkages. There are several arrangements of the pentose constituents which would satisfy the above requirements. The simplest is that given in (XXI). Here the three acid labile side chains (A) are represented as being each composed of three arabofuranose units. One of these side chains (A) is joined to that galactose unit of the main chain which is not involved in the branching of the nucleus of degraded mesquitic acid. The other two side chains (A) are joined as shown to those galactose units which form part of the aldobiuronic acid residues.

The structure (XXI) used for purposes of discussion shows three galactopyranose units in

the main chain united by 1 : 6-linkages, and to position 3 of each of these units a side chain is attached. These side chains, three in number, are composed of two residues of aldobionic acid and one (A) of arabofuranose units. It will be apparent, however, that it is also possible for the three galactose units to be joined by 1 : 3-linkages while the side chains are attached to position 6 of each of the three galactose units in the main chain. Such a modification of the union of the 3 galactose units in the main chain would still be in conformity with the established fact that these galactose units give rise to 2 : 4-dimethyl galactose.

Furthermore, the possibility of the three galactose units of the main chain being mutually joined by 1 : 6- and 1 : 3-linkages cannot be excluded. In such a case, the side chain (A) and the two aldobionic acid side chains would be attached to position 3 of the galactose units already linked in the main chain through position 6, and to position 6 of the galactose units which have position 3 involved in the main chain linkage (White, *J. Amer. Chem. Soc.*, 1947, **69**, 622). Substantial evidence as to the precise mode of linkage of the galactose constituents of the main chain will best be obtained by the methylation of degraded mesquitic acid [*i.e.*, of mesquitic acid from which the labile arabofuranose side chains (A) have been removed by acid hydrolysis] followed by a study of the partly methylated products of hydrolysis. A preliminary study of this methylated degraded mesquitic acid was made in 1939, but owing to the war the completion of the examination had to be postponed; this will form the subject of a later communication.

In mesquite gum, as in gum arabic, damson gum, and cherry gum, the complex structures are composed of several kinds of sugars joined by different types of glycosidic bonds. Both hexoses and pentoses are found as members of polysaccharide gums, and it is of particular interest that hexoses and their derivatives—for example, rhamnose, fucose, glucuronic acid, and galacturonic acid—occur only in pyranose form (Hirst, *J.*, 1942, 77). Of the pentoses, arabinose seems to exist only in the furanose form as in the gums and arabans, while xylose occurs for example in xylan (Haworth, Hirst, and Oliver, *J.*, 1934, 1917), gum tragacanth (Luckett and Smith, *J.*, 1945, 739), damson gum (Hirst, *loc. cit.*), and the mucilage of *Plantago lanceolata* (Mullan and Percival, *J.*, 1940, 1501) in the pyranose form. It is also curious that L-arabinose and D-glucuronic acid often form constituents of the same molecular complex, as in the gum under review, gum arabic (Smith, *J.*, 1940, 1035), and cherry and damson gums (Hirst, *loc. cit.*), while D-galacturonic acid and D-xylose have been identified as constituents of gum tragacanth (Luckett and Smith, *loc. cit.*). Seldom does D-galacturonic acid occur with L-arabinose, and, although D-glucuronic acid and D-xylose form members of the same complex as in damson gum (Hirst and Jones, *J.*, 1938, 1174), the proportion of xylose is small, and furthermore there is present also a much larger proportion of L-arabinose. Glucose still remains to be identified as a constituent of plant gums. The significance of these facts is not yet clear, but they seem to indicate that in the plant the formation of a pentose from a hexose, if it occurs, does not involve the intermediate formation and decarboxylation of a uronic acid. For example, D-galacturonic acid could not be intermediate in the formation of L-arabofuranose units from D-galactopyranose unless a change from a pyranose to a furanose ring structure took place analogous to that which occurs with remarkable ease in the acid-catalysed conversion of 3 : 6-anhydro-α-methylglucopyranoside into 3 : 6-anhydro-α-methylglucofuranoside (Haworth, Owen, and Smith *J.*, 1941, 88). Nevertheless, the fact remains that oxidation or reduction of terminal groups of hexoses does occur in Nature as is shown by the inter-relationship of D-glucose, D-glucuronic acid, and D-xylose; of D-galactose, D-galacturonic acid, and L-arabinose; of D-galactose and L-fucose; and of D-mannose and L-rhamnose.

Experimental.

Methylation of Mesquitic Acid.—Mesquitic acid (20 g.) which had equiv. wt. of 1300 (by titration with sodium hydroxide) and 1290 (from uronic anhydride content of 13·7%) was methylated by dissolving it in sodium hydroxide (200 c.c. of a 30% solution) and gradually adding methyl sulphate (250 c.c.) and sodium hydroxide (500 c.c. of a 30% solution) during 4 hours at room temperature with vigorous stirring, which was continued for a further 8 hours after the addition of all the methyl sulphate. The resulting solution was cooled in an ice-bath, almost neutralised with dilute sulphuric acid, and filtered to remove sodium sulphate, the filtrate concentrated to a suitable volume (100—150 c.c.) under reduced pressure, and the methylation repeated using methyl sulphate (250 c.c.) and sodium hydroxide (700 c.c. of a 30% solution). During the methylation, acetone was added as required to keep the methylated material in solution. After the fifth methylation the sodium salt of the methylated polysaccharide, which separated readily upon completion of the methylation by heating for 15—20 minutes on the boiling water-bath, was dissolved in aqueous acetone (60 c.c. water, 40 c.c. acetone) and treated with methyl sulphate (250 c.c.) and sodium hydroxide (750 c.c. of a 30% solution) at 35°, the reagents being added gradually during 2½ hours. The methylation was completed by heating the solution for 15 minutes on the boiling water-bath, whereby the excess of acetone was expelled and the sodium salt of methylated mesquitic acid separated on the surface of the mixture as yellow nodules. These were removed by filtration while

the solution was still hot, since the sodium salt of methylated mesquitic acid was less soluble in hot than in cold water. After two more methylations at 35° a portion of the sodium salt of methylated mesquitic acid was suspended in water, acidified with dilute sulphuric acid, and extracted with chloroform. After being washed with sodium sulphate solution (20%) and dried ($MgSO_4$), the chloroform solution was concentrated under reduced pressure to give a glassy solid, which after dissolution in acetone, was poured into excess of light petroleum. This precipitated the methylated polysaccharide as a white amorphous powder which after being dried in a vacuum at 60° had $[\alpha]_D^{20°}$ + 60° in chloroform (*c*, 1·3) (Found: OMe, 39·1%; equiv., 1540). The remainder of the sodium salt of methylated mesquitic acid was subjected to 3 further methylations, and the product (8 g.) was purified as above (Found: OMe, 39·0%). Three treatments of mesquite gum with methyl sulphate and sodium hydroxide are probably enough to give complete methylation.

The Acetate of Mesquitic Acid.—Freshly prepared mesquitic acid (100 g.) (Cunneen and Smith, *loc. cit.*) was dissolved in pyridine (1 l.), and acetic anhydride (1 l.) gradually added during 2 hours. After 2 more hours, the solution was poured slowly with stirring into water (10 l.), the precipitated mesquitic acetate was washed 3 times with water, filtered off, and washed once with ethyl alcohol and finally with ether. After being dried in a vacuum at 45° it was obtained as a fine white amorphous powder (114 g.).

Methylation of the Acetate of Mesquitic Acid.—Mesquitic acetate was methylated in portions of 20 g. by dissolving each portion in aqueous acetone (200 c.c. acetone, 100 c.c. water) and gradually adding sodium hydroxide (700 c.c. of a 30% solution) and methyl sulphate (250 c.c.) during 1½ hours at 45°. The methylation was completed by heating the mixture at 90° for 20 minutes to remove all acetone. The sodium salt of the methylated mesquitic acid separated as yellow nodules which were removed by filtration and subjected to a further 5 methylations. After this treatment the sodium salt of the partially methylated gum was suspended in water (500 c.c.) and acidified with dilute sulphuric acid, and the solution extracted 3 times with chloroform. Evaporation of the chloroform solution, after it had been washed with a concentrated solution (*ca.* 20%) of sodium sulphate and dried (Na_2SO_4), yielded the methylated polysaccharide (17 g.) which had $[\alpha]_D^{18°}$ + 62° in chloroform (*c*, 1·0) (Found: OMe, 39·0%; equiv., 1625).

Methylation was completed by boiling a solution of the polysaccharide in methyl iodide (30 c.c.) under reflux for 6 hours, during which time silver oxide (15 g.) was gradually added. Following removal of the excess of methyl iodide by distillation the methylated product was extracted with acetone; evaporation of the acetone solution gave the methylated gum as a glassy solid (16 g.) which was subjected to three further methylations with Purdie's reagents (silver oxide and methyl iodide).

Fractionation of Methylated Mesquitic Gum.—To a solution of the methylated polysaccharide (32 g.) in ether (250 c.c.), light petroleum (b. p. 40—60°) was added slowly with stirring to give a precipitate (fraction 1). The mother liquor was decanted and from it two more fractions (2 and 3) were successively precipitated with light petroleum. Fraction 4 was obtained as a white amorphous powder by pouring the solution, left after the precipitation of the first 3 fractions, into excess of light petroleum. Each fraction was then redissolved in ether (50 c.c.) and reprecipitated by pouring the solution into excess of light petroleum. The properties of these fractions after being dried in a vacuum are given in the Table. Fractions 2 and 3 showed almost identical properties and were accordingly subjected to hydrolytic examination.

Fraction.	Wt (g.).	OMe (%).	$[\alpha]_D^{20°}$ ($CHCl_3$).	Ash (%).	Equiv.	η in *m*-cresol (*c*, 0·46).
1	4	40·1	44°	0·4	1320	0·323
2	10	40·0	55	nil	1525	0·152
3	9	40·25	56	nil	1542	0·135
4	2·5	40·2	64	nil	1385	0·235

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment I.—In this preliminary experiment the methylated polysaccharide (9·4 g.) was boiled for 6 hours with methyl alcohol (350 c.c.) containing 2% of dry hydrogen chloride. The solution was neutralised with silver carbonate and filtered, the residue being repeatedly washed with hot methyl alcohol. The combined filtrate and washings were evaporated under reduced pressure, and a syrup (9·2 g.) remained which was dissolved in water (100 c.c.) and heated for 2 hours at 60° with barium hydroxide (1·5 g.). Excess of alkali was neutralised with carbon dioxide, the mixture heated for 15 minutes at 85° to decompose any barium hydrogen carbonate and filtered, and the precipitates washed with hot water. The filtrate was evaporated to a small volume (50 c.c.) and extracted 10 times with chloroform (300 c.c.); and the dried chloroform solution was filtered and concentrated to give a mobile mixture of methylated glycosides (*A*) (5·25 g.). The aqueous solution left after the chloroform extraction furnished on evaporation a syrup consisting of the barium salts of the methylated uronic and aldobionic acids contaminated with some methylated glycosides. The latter were removed by exhaustive extraction with ether; evaporation of this ethereal extract gave syrupy glycosides (*B*) (0·9 g.) which partially crystallised. The residual barium salt (*C*) amounted to 4·2 g.

Examination of the Methylated Glycosides (A).—Distillation of the methylated glycosides gave:

Fraction.	Wt. (g.).	B.p. (bath temp.).	Pressure (mm.).	$n_D^{18°}$.	OMe (%).
(I)	1·6	137—140°	0·05	1·4365	59·0
(II)	3·0	150—180	0·02	1·4465	48·8
(III)	0·39	180—200	0·02	1·4565	45·3

Identification of 2 : 3 : 5-*Trimethyl Methylarabinoside* (I).—When a solution of fraction (I) in 0·2N-sulphuric acid (50 c.c.) was heated on the boiling water-bath for 7 hours, the rotation changed from $[\alpha]_D^{18°}$ − 58° (initial value) to − 43° (constant value). The acid was neutralised with barium

carbonate, and the solution filtered and evaporated to dryness. Extraction of the residue with ether gave a syrup (0·96 g.) consisting of 2 : 3 : 5-trimethyl arabinose (II) which had $[\alpha]_D^{20°}$ − 37° in water (*c*, 0·65), n_D^{17} 1·4555 (Found : OMe, 44·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

Oxidation with bromine. The syrup was dissolved in water (10 c.c.) and treated with bromine (1·5 c.c.) for 48 hours at room temperature. The bromine was removed by aeration, and the non-reducing solution neutralised with silver carbonate and filtered before and after passing in hydrogen sulphide. The clear solution was evaporated to dryness and extracted with dry ether; concentration of the extracts gave 2 : 3 : 5-trimethyl γ-arabonolactone (III), b. p. 120° (bath temp.)/0·024 mm., $n_D^{19°}$ 1·4465 (0·7 g.), which crystallised on nucleation and had $[\alpha]_D^{17°}$ − 47° (initial value) in water (*c*, 1·0) changing to $[\alpha]_D^{17°}$ − 36° (constant value) in 20 days (Found : OMe, 46·6. Calc. for $C_8H_{14}O_5$: OMe, 48·9%).

2 : 3 : 5-*Trimethyl* L-*Arabonamide* (IV).—Treatment of the 2 : 3 : 5-trimethyl γ-arabonolactone with methyl-alcoholic ammonia at 0° for 2 days followed by removal of the solvent under reduced pressure afforded the crystalline amide of 2 : 3 : 5-trimethyl L-arabonic acid, m. p. and mixed m. p. 138°, $[\alpha]_D^{16°}$ + 18° in water (*c*, 1·3) after crystallisation from acetone–ether (Found : OMe, 44·6. Calc. for $C_8H_{17}O_5N$: OMe, 44·9%).

Identification of 3 : 5-*Dimethyl Methyl-*L-*Arabinoside* (V).—A portion of fraction (II) (0·6 g.) after being methylated twice with Purdie's reagents in the usual way gave a syrupy 2 : 3 : 5-trimethyl methylarabinoside (0·45 g.) (Found : OMe, 59·0. Calc. for $C_9H_{18}O_5$: OMe, 60·25%), recognised by its conversion into the crystalline 2 : 3 : 5-trimethyl γ-arabonolactone which the corresponding 2 : 3 : 5-trimethyl arabinose furnished on oxidation with bromine. Treatment of this lactone with methanolic ammonia yielded 2 : 3 : 5-trimethyl L-arabonamide, m. p. and mixed m. p. 138°, $[\alpha]_D^{18°}$ + 15° in water (*c*, 1·4) (Found : OMe, 44·7. Calc. for $C_8H_{17}O_5N$: OMe, 44·9%).

3 : 5-*Dimethyl Arabinose* (VI).—When a solution of fraction (II) (1·0 g.) in 0·1N-sulphuric acid (50 c.c.) was heated on the boiling water-bath it showed $[\alpha]_D^{19°}$ − 87° (initial value), − 56° (after 2 hours), − 50° (3 hours), − 45° (4 hours), − 45° (8 hours) (constant value). The concentration of acid was increased to 1N and the solution heated for 4 hours, but no further change in the rotation was observed. The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under diminished pressure. Extraction of the residue with alcohol followed by filtration and removal of solvent yielded 3 : 5-dimethyl arabinose (VI) (Found : OMe, 35·7. Calc. for $C_7H_{14}O_5$: OMe, 34·8%).

3 : 5-*Dimethyl γ-Arabonolactone* (VII).—After treatment of a solution of the dimethyl sugar (0·6 g.) in water (2 c.c.) with bromine (0·6 c.c.) for 48 hours at room temperature, the solution, after removal of the excess of bromine, no longer reduced Fehling's solution. The lactone, isolated in the usual way, gave on distillation a colourless liquid, b. p. 135° (bath temp.)/0·025 mm. (0·35 g.), $n_D^{20°}$ 1·4580, which crystallised spontaneously. The crystals of 3 : 5-dimethyl γ-arabonolactone, after recrystallisation from ether, had m. p. 75°, $[\alpha]_D^{17°}$ − 84·5° (initial value in water, *c*, 1·5), − 84° (after 3 hours), − 79° (10 hours), − 73° (20 hours), − 71° (25 hours), − 69° (28 hours), − 69° (50 hours) (Found : C, 47·35; H, 7·2; OMe, 35·3. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·9; OMe, 35·2%).

3 : 5-*Dimethyl* L-*Arabonamide* (VIII).—Treatment of the lactone (0·25 g.) with dry methyl-alcoholic ammonia for 24 hours at 0° gave the amide, which after removal of solvent and crystallisation from acetone had m. p. 145°, $[\alpha]_D^{18°}$ + 10° in water (*c*, 2·6) (Found : OMe, 32·1; N, 7·1. Calc. for $C_7H_{15}O_5N$: OMe, 32·1; N, 7·25%).

A portion of the amide (9 mg.) was dissolved in water (0·2 c.c.) and a solution of 1·5N-sodium hypochlorite (0·2 c.c.) added. The mixture was kept at 0° for ½ hour and the excess of hypochlorite destroyed by the addition of 2 drops of sodium thiosulphate solution. Addition of sodium acetate (0·5 g.) to the solution, followed by semicarbazide hydrochloride, gave in 2—3 minutes a white precipitate of hydrazodicarbonamide, m. p. and mixed m. p. 256° (decomp.); yield 70%.

Fraction (III).—This fraction which had $[\alpha]_D^{18°}$ − 35° in water (*c*, 1·2) appeared to be a mixture of 3 : 5-dimethyl methylarabinoside and 2 : 4-dimethyl methylgalactoside and was not further examined (Found : OMe, 45·3%).

Examination of the Methylated Glycosides (B). *Identification of* 2 : 4-*Dimethyl β-Methylgalactoside* (IX).—The partly crystalline glycosides (*B*) furnished on distillation :

Fraction.	Wt. (g.).	B.p. (bath temp.) at 0·02 mm.	$n_D^{16·5°}$.	OMe (%).
(IV)	0·12	138—150°	1·4575	44·4
(V)	0·71	160—165	1·4670	40·0

Examination of Fraction (IV).—This fraction had $[\alpha]_D^{18°}$ − 28·4° in water (*c*, 1·27), and like fraction (III) appeared to be a mixture of 3 : 5-dimethyl methylarabinoside and 2 : 4-dimethyl methylgalactoside.

Trituration of the crystalline fraction (V) with acetone and ether gave 2 : 4-dimethyl β-methylgalactoside (IX) which, after 3 crystallisations from acetone–ether, had m. p. and mixed m. p. 167°; $[\alpha]_D^{13°}$ + 2·0° in water (*c*, 1·0) (Found : C, 48·3; H, 7·7; OMe, 41·25. Calc. for $C_9H_{18}O_6$: C, 48·6; H, 8·2; OMe, 41·9%).

When a solution of a portion (0·36 g.) of the crystalline galactoside in N-sulphuric acid (10 c.c.) was boiled it showed the following rotations : $[\alpha]_D^{16°}$ + 2·0° (initial value); + 31° (after 1 hour); + 91° (5 hours); + 88° (10 hours) (constant value). The solution was neutralised with barium carbonate, filtered, and evaporated to dryness under reduced pressure to give the free sugar (0·3 g.) which was purified by extraction with ethyl alcohol followed by removal of the solvent.

2 : 4-*Dimethyl Galactose Anilide.*—A portion (0·15 g.) of the dimethyl sugar was boiled with alcohol (5 c.c.) containing aniline (0·07 g.) for 3 hours; the alcohol was distilled off and the anilide crystallised on cooling. The crystals of 2 : 4-dimethyl galactose anilide had m. p. 214° after recrystallisation from ethyl alcohol. It gave no depression when in admixture with an authentic specimen.

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (XI).—The ether-insoluble barium salts (*C*) (4·2 g.) were dissolved in methyl alcohol (200 c.c.) containing 1% of hydrogen chloride and left for 2 days at room temperature. The solution was neutralised with silver carbonate and filtered.

Evaporation of the filtrate gave a syrup (2·5 g.) (Found : OMe, 47·0%) consisting of the methyl esters of the methylated uronic and aldobiuronic acids and probably some dimethyl methylgalactoside which upon fractional distillation gave :

Fraction.	Wt. (g.).	B. p. (bath temp.).	$n_D^{22°}$.	OMe (%).	Equiv.
(VI)	0·5	160—165°/0·01 mm.	1·4507—1·4595	49·6	353
(VII)	1·3	225—270/0·01 mm.	1·4705—1·4735	47·6	467

There remained a residue of 0·7 g.

Fraction (VI).—This fraction (OMe, 49·6%; equiv., 353) was probably a mixture of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (OMe, 58·6%; equiv., 264) and 2 : 4-dimethyl methylgalactoside (OMe, 41·9%). The latter was not sufficiently soluble in ether to be completely removed by the ether extraction procedure which was applied above for the separation of the mixture of barium salts and methylated glycosides.

The presence of the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside in fraction (VI) was proved by boiling the latter (0·3 g.) for 6 hours with 3% methyl-alcoholic hydrogen chloride (20 c.c.). The cooled solution was neutralised with silver oxide, filtered, and evaporated to dryness. Purification of the syrupy residue by extraction with ether followed by distillation in a vacuum gave a colourless liquid containing the methyl ester of 2 : 3 : 4-trimethyl α-methylglucuronoside (Smith, *J.*, 1939, 1724). Treatment of this distillate with methyl-alcoholic ammonia for 2 days at 0° followed by removal of the excess of solvent gave a crystalline residue from which the amide (XII) of 2 : 3 : 4-trimethyl α-methylglucuronoside was separated by crystallisation from ethyl alcohol–light petroleum; m. p. 184° alone or in admixture with an authentic specimen.

Fraction (VII).—This fraction, which had $n_D^{18°}$ 1·4735, $[\alpha]_D^{17°}$ + 47° in water (*c*, 0·5), appeared to be the methyl ester of hexamethyl aldobiuronic acid (Found : OMe, 47·6; equiv., 467. Calc. for $C_{19}H_{34}O_{12}$: OMe, 47·8%; equiv., 454).

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment II.—This experiment was carried out in order to secure enough of the methylated aldobiuronic acid [see fraction (VII) above] for purposes of identification and with the object of estimating the relative quantities of the different hydrolytic cleavage fragments.

The methylated mesquitic gum (20 g.) was hydrolysed by boiling it for 6 hours with 2% methyl-alcoholic hydrogen chloride (320 c.c.); the specific rotation then changed from + 57° to − 18°. The mixture was cooled, neutralised with silver carbonate, and filtered. After the residue had been washed several times with hot methyl alcohol, the combined filtrate and washings were evaporated to dryness under reduced pressure. The syrupy residue (20 g.) was heated for 2 hours at 60° with barium hydroxide (2·5 g.) in water (200 c.c.). The excess of barium hydroxide was neutralised with carbon dioxide and the solution was heated on the boiling water-bath for 15 minutes to decompose barium hydrogen carbonate. The solution was filtered and evaporated to dryness under reduced pressure. Six extractions of this pale yellow syrup with ether gave a mixture of ether-soluble glycosides (*D*) (11·7 g.), and there remained the ether-insoluble barium salt (*E*).

Fractional Distillation of the Glycosides (D).—Slow distillation of the mixture through a narrow 4-inch vacuum jacketed column yielded : *fraction* (a), 2 : 3 : 5-trimethyl methyl-L-arabinoside (3·1 g.), b. p. 115—125° (bath temp.)/0·17 mm., $n_D^{19°}$ 1·4380, $[\alpha]_D^{17°}$ − 56° in water (*c*, 1·2) (Found : OMe, 59·2%); *fraction* (b), 3 : 5-dimethyl methyl-L-arabinoside (6·3 g.), b. p. 130—135° (bath temp.)/0·1 mm., $n_D^{19°}$ 1·4475, $[\alpha]_D^{20°}$ − 66° in water (*c*, 1·3) (Found : OMe, 48·5%); *fraction* (c), 2 : 4-dimethyl β-methylgalactoside (1·8 g.), b. p. 160—180° (bath temp.)/0·033 mm., $[\alpha]_D^{18°}$ + 2° in water (*c*, 0·8), m. p. and mixed m. p. 168° (Found : OMe, 41·7%). The residue (0·52 g.) in the distilling flask crystallised on cooling, and recrystallisation showed that it was a mixture of the α and the β form of 2 : 4-dimethyl methylgalactoside.

Examination of the Barium Salts (E).—The ether-insoluble barium salts (*E*) were converted into the corresponding methyl esters by treatment with methyl alcohol containing 2% of dry hydrogen chloride for 2 days at room temperature. After neutralisation with silver carbonate the filtered solution gave, on removal of solvent, a syrupy product (5·0 g.) which upon fractional distillation yielded : *fraction* (d) (0·9 g.), b. p. 155—190° (bath temp.)/0·02 mm., $n_D^{19°}$ 1·4565; *fraction* (e) (2·1 g.), b. p. 230—265° (bath temp.)/0·02 mm., $n_D^{19°}$ 1·4770 (Found : OMe, 46·5; equiv., 474. Calc. for $C_{19}H_{34}O_{12}$: OMe, 47·8%; equiv., 454).

The *residue* (F) (2·9 g.) which remained in the flask was examined later.

Identification of the Methyl Ester of 2 : 3 : 4-*Trimethyl Methylglucuronoside* (XI).—Redistillation of fraction (*d*) gave the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (0·7 g.), b. p. 115—120° (bath temp.)/0·03 mm., $n_D^{19°}$ 1·4495 (Found : OMe, 56·3; equiv., 284. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264). A crystalline residue of almost pure 2 : 4-dimethyl β-methylgalactoside (0·2 g.) remained.

Treatment of the ester with methyl-alcoholic ammonia at 0° gave the amide of 2 : 3 : 4-trimethyl α-methylglucuronic acid which after crystallisation from ethyl alcohol–ether–light petroleum had m. p. and mixed m. p. 185°, $[\alpha]_D^{18°}$ + 138° in water (*c*, 0·53) (Found : C, 48·5; H, 7·7; N, 5·6; OMe, 49·4. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·7; N, 5·6; OMe, 49·8%).

Identification of the Methyl Ester [*Pentamethyl Methylaldobiuronoside* (XIII)] *of the Hexamethyl Aldobiuronic Acid.*—A solution of *fraction* (e) (2·0 g.) in dry methyl alcohol (100 c.c.) containing 8% of dry hydrogen chloride was boiled for 22 hours; the specific rotation had then changed from $[\alpha]_D^{17°}$ + 80° to $[\alpha]_D^{17°}$ + 69°. The solution was neutralised with silver carbonate and filtered, and the residue washed well with methyl alcohol. Evaporation of the filtrate gave a syrup (2·0 g.) which on fractional distillation yielded : *fraction* (f) (0·59 g.), b. p. 112—120° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4520 (Found : OMe, 58·1; equiv., 275. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264); *fraction* (g) (0·61 g.), b. p. 145—165° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4720 (Found : OMe, 42·2. Calc. for $C_9H_{16}O_6$: OMe, 41·9%) (this fraction crystallised spontaneously); *fraction* (h) (0·45 g.), b. p. 165—170° (bath temp.)/0·025 mm., $n_D^{18°}$ 1·4810. A *residue* (G) (0·35 g.) remained in the distilling flask.

Examination of Fraction (f).—This fraction was identified as the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI), since treatment with methyl-alcoholic ammonia furnished, in good yield, the crystalline amide (XII) of 2 : 3 : 4-trimethyl α-methylglucuronoside, m. p. 185°, $[\alpha]_D^{18°}$ + 133° in water (*c*, 0·6) (Found : C, 48·5; H, 7·6; N, 5·6; OMe, 48·7. Calc. for $C_{10}H_{19}O_6N$: C, 48·2; H, 7·75; N, 5·6; OMe, 49·8%).

Examination of Fraction (g).—After recrystallisation from acetone–ether the crystalline fraction gave 2 : 4-dimethyl β-methylgalactoside (IX), $[\alpha]_D^{16°}$ + 4° in water (*c*, 0·8), m. p. and mixed m. p. 166°. Hydrolysis of a small portion of fraction (*g*) by heating it on a boiling water-bath with N-sulphuric acid for 12 hours followed by neutralisation with barium carbonate gave 2 : 4-dimethyl galactose (X) which crystallised readily as the monohydrate on the addition of a little water; m. p. and mixed m. p. 102° (after recrystallisation from ethyl alcohol–acetone–ether). Treatment of anhydrous 2 : 4-dimethyl galactose with boiling ethyl-alcoholic aniline gave crystalline 2 : 4-dimethyl galactose anilide which separated from the mixture, m. p. 214°, mixed m. p. 216°.

Examination of Fraction (h).—This fraction like the distillation residue (*G*) appeared to be the methyl ester of a hexamethyl aldobionic acid (Found : OMe, 46·4; equiv., 460. Calc. for $C_{18}H_{34}O_{12}$: OMe, 47·8%; equiv., 454). This methyl ester of the methylated aldobionic acid is relatively stable, since further treatment of fraction (*h*) with boiling methyl alcohol containing 8% of dry hydrogen chloride for 25 hours effected no further hydrolysis.

Further Hydrolysis of the Residue (F).—A solution of this syrupy residue (*F*) (2·9 g.) in methyl alcohol (30 c.c.) containing 8% of dry hydrogen chloride was boiled for 12 hours. The solution was neutralised with silver carbonate, filtered, and evaporated to dryness under reduced pressure. The syrupy residue was purified by extraction with a mixture of acetone (1 part) and ether (5 parts). Distillation of the syrup gave : *fraction* (i) (0·2 g.), b. p. 132—145° (bath temp.)/0·04 mm., $n_D^{21°}$ 1·4505 (Found : equiv., 270); *fraction* (j) (1·3 g.), b. p. 155—165° (bath temp.)/0·05 mm. (Found : OMe, 41·3%). A *residue* (H) (1·4 g.) remained in the distillation flask and had $n_D^{21°}$ 1·4820 (Found : OMe, 46·2%; equiv., 485).

Examination of Fraction (i).—This was the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside (XI) and was characterised by its conversion into the crystalline amide, m. p. and mixed m. p. 185°, in the manner already described.

Examination of Fraction (j).—This crystalline fraction was 2 : 4-dimethyl β-methylgalactoside (IX) and had m. p. and mixed m. p. 167° (after recrystallisation from acetone–ether).

Examination of the Residue (H).—This was the hexamethyl aldobionic acid characterised by its resistance towards hydrolysis.

Hydrolysis of the Residues (H) *and* (G) *and Fraction* (h) *Consisting of the Resistant Hexamethyl Aldobionic Acid.*—The residue (*H*) was combined with the residue (*G*) and fraction (*h*) since all these fractions had proved resistant towards hydrolysis by 8% methyl-alcoholic hydrogen chloride.

A portion (0·52 g.) of this mixture (2·2 g.) was dissolved in methyl alcohol (20 c.c.) containing 2% of dry hydrogen chloride and heated in a Carius tube at 130° for 2 days. The solution was neutralised with silver carbonate and filtered, and the filtrate evaporated to yield a syrup (0·5 g.) which on fractional distillation gave : *fraction* (k) (the methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside) (0·22 g.), b. p. 110—135° (bath temp.)/0·02 mm., $n_D^{20°}$ 1·4515 (Found : OMe, 57·5; equiv., 281. Calc. for $C_{11}H_{20}O_7$: OMe, 58·6%; equiv., 264); *fraction* (l) a dimethyl methyl(?)galactoside (0·25 g.), b. p. 160—210° (bath temp.)/0·02 mm., $n_D^{20°}$ 1·4745 (Found : OMe, 41·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). This fraction (*l*) failed to crystallise. It is believed that it consists of a dimethyl methylgalactoside and probably arises from the methylated aldobiuronic acid (?) (XX), stable to 8% methyl-alcoholic hydrogen chloride, which possesses the 1 : 4-glycosidic linkage. For this reason the dimethyl methylgalactoside is probably a 2 : 3, 2 : 6, or a 3 : 6 derivative. It will be examined further.

The results of this second hydrolysis of the methyl ester of methylated mesquitic acid (20 g.) were as follows :

Compound.	Wt. (g.).	*M*.	Wt. × 100, mols.	Molar prop. (approx.).
2 : 3 : 5-Trimethyl methylarabinoside	3·1	206	1·51	3
3 : 5-Dimethyl methylarabinoside	6·3	192	3·28	6—7
2 : 4-Dimethyl methylgalactoside	4·43	222	2·0	4
Methyl ester of 2 : 3 : 4-trimethyl methylglucuronoside	2·46	264	0·93	2
Unknown dimethyl methylgalactoside	1·1	222	0·495	1

Although a considerable loss of material has occurred this was not unexpected in view of the extensive handling of the products, and it is believed that the above relative proportions of the cleavage fragments are approximately correct.

Hydrolysis of the Methyl Ester of Methylated Mesquitic Acid. Experiment III.—A solution of the methyl ester of methylated mesquitic acid (12 g.) in 0·5% methyl-alcoholic hydrogen chloride (300 c.c.) was boiled under reflux, during which the following changes occurred : $[\alpha]_D$ + 53° (initial value); + 49° (after 0·5 hours); + 44° (1 hour); + 36° (2 hours); + 22° (4 hours); + 9° (7 hours); + 7° (9 hours); + 1·5° (13 hours). The hydrolysis was arrested at this point and the solution was neutralised with silver carbonate, filtered, and concentrated to a syrup (12 g.). This was dissolved in water (100 c.c.) containing barium hydroxide (1·6 g.) and the solution heated for 2 hours at 60—70°. The excess of barium hydroxide was neutralised with carbon dioxide and the solution filtered and evaporated to dryness under reduced pressure. The dry residue consisting of the barium salts of the methylated acids and the methylated glycosides was extracted 6 times with ether under reflux. Removal of the solvent from the ethereal solution gave a mixture of the methylated glycosides (3·8 g.). There remained an ether-insoluble residue of barium salts (4·2 g.).

Examination of the Ether-insoluble Barium Salts.—The barium salts (4·2 g.) were esterified by treatment with 1% methyl-alcoholic hydrogen chloride (150 c.c.) for 2 days at room temperature. The solution was then neutralised with silver carbonate, filtered, and concentrated to give the methyl ester

as a glassy residue (2·86 g.) [Found : equiv., 760. Calc. for the methyl ester of a degraded mesquitic acid (in the form of a methyl glycoside) composed of 5 units of dimethyl galactose and 2 units of 2 : 3 : 4-trimethyl glucuronic acid : equiv., 723].

Further Hydrolysis of the Methyl Ester of Methylated Degraded Mesquitic Acid.—When a solution of the methyl ester (2·86 g.) in 1% methyl-alcoholic hydrogen (100 c.c.) was boiled, the following changes were observed : $[\alpha]_D$ + 35° (initial value) ; + 33·5° (after 1 hour) (constant for a further 3 hours). Since no hydrolysis seemed to have taken place the concentration of the hydrogen chloride in the methyl-alcoholic solution was increased to 2% by the addition of the requisite amount of 10% methyl-alcoholic hydrogen chloride. The mixture was boiled again and the following changes were observed : $[\alpha]_D$ + 33·5° (initial value) ; + 42·5° (after 2 hours) ; + 45° (4 hours) (constant for 1 hour).

The solution was then neutralised with silver carbonate, filtered, and concentrated to give a syrupy mixture consisting of methyl esters and methylated glycosides (2·7 g.) which was treated with barium hydroxide (1·0 g.) in water (50 c.c.) for 2 hours at 60°. The excess of barium hydroxide was neutralised with carbon dioxide and the solution was filtered and evaporated to dryness under reduced pressure. The mixture of barium salts and methylated glycosides thus produced was exhaustively extracted with dry ether under reflux. Concentration of the ethereal solution gave a syrup (0·95 g.) which on being kept afforded crystalline 2 : 4-dimethyl β-methylgalactoside (IX), m. p. and mixed m. p. 167° (Found : OMe, 41·0. Calc. for $C_9H_{18}O_6$: OMe, 41·9%). The ether-insoluble barium salts were dissolved in 1% methyl-alcoholic hydrogen chloride (50 c.c.) and left for several days at room temperature in order to effect esterification without hydrolysis. The solution was neutralised with silver carbonate, filtered, and concentrated to a syrup. This was extracted with dry ether under reflux, and removal of the solvent gave the methyl esters as a syrup (1·24 g.)

From this hydrolsysis of methylated mesquitic gum it will be seen that 2% methyl-alcoholic hydrogen chloride is necessary to hydrolyse the methyl ester of the methylated degraded mesquitic acid. In addition 0·5% and 1% methyl-alcoholic hydrogen chloride appear to effect cleavage of the arabinose residues from the polysaccharide molecule, leaving the methylated degraded mesquitic acid. The latter will be studied further.

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[*Received, June 12th*, 1947.]

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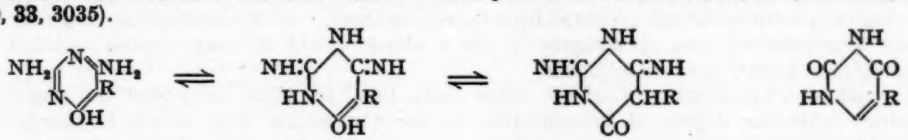
229. *Some 2 : 4-Diamino-5-acylamido-6-hydroxypyrimidines.*

By WALTER WILSON.

Several 5-acylamides have been made from 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2) either by using an acid chloride or anhydride, or by direct condensation with a carboxylic acid. For example, acetic anhydride gave 2 : 4-*diamino-5-acetamido-6-hydroxypyrimidine* (I; R = NHAc). The structure of this was established by an independent synthesis from ethyl acetamidocyanoacetate. Direct proof of the preferential acylation of the 5 amino-group in (I; R = NH_2) was thus obtained. The structure of xanthopterin (IV) is confirmed by these results. The reactions of pyrimidines of the types studied are best represented by the formula (I*A*) or (I*B*), which also agree with the light-absorption data. 2 : 4-*Diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* was less active than sulphanilamide against a number of bacteria.

IN the course of work on compounds related to folic acid, several 2 : 4-diamino-5-acylamido-6-hydroxypyrimidines have been prepared. A few acyl derivatives of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2), which were believed to be the 5-acylamides, had been obtained previously by heating the pyrimidine with carboxylic acids at temperatures from 80° to 140° (Bayer, G.PP. 206,454, 213,711; B.P. 15,573; Traube and Dudley, *Ber.*, 1913, **46**, 3839; Purrmann, *Annalen*, 1940, **546**, 98; Wellcome, B.P. 579,138). Amino-groups in positions 2 and 4 of highly substituted amino-hydroxy-pyrimidines are often unreactive, whereas those in the 5 position acylate normally and react with cyanic acid (*e.g.*, Isay, *Ber.*, 1906, **39**, 257; Gabriel and Coleman, *Ber.*, 1901, **34**, 1236; Levene and Senior, *J. Biol. Chem.*, 1916, **25**, 607; Traube, *Ber.*, 1900, **33**, 3035).



(I.) (I*A*.) (I*B*.) (II.)

Treatment of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I; R = NH_2) with acetic anhydride and aqueous alkali gave 2 : 4-*diamino-5-acetamido-6-hydroxypyrimidine* (I; R = NHAc) which formed a stable *picrate*. The remaining amides described here did not form picrates, although with the exception of the oxamic acid they were soluble in dilute mineral acids.

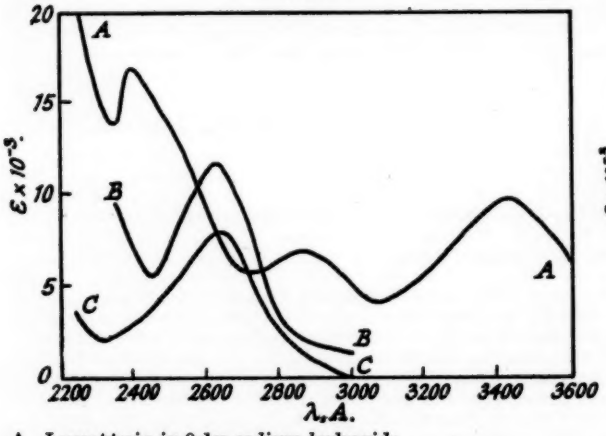
The 5-acetamido-compound has also been made by condensing guanidine with ethyl

acetamidocyanoacetate, a method which established unequivocally the preferential acetylation of the 5-amino-group.

The pyrimidine (I; R = NH_2) gave 2 : 4-*diamino-5-benzamido-6-hydroxypyrimidine* (I; R = NHBz) with benzoyl chloride and aqueous alkali. Two other amides were prepared from the pyrimidine by interaction with carboxylic acids under suitable conditions (cf. Bayer *et al.*, *loc. cit.*). Thus the pyrimidine (as the sodium hydrogen sulphite compound) with lactic acid at 115—125° yielded 2 : 4-*diamino-5-α-hydroxypropionamido-6-hydroxypyrimidine* (I; R = $NH{\cdot}CO{\cdot}CH(OH){\cdot}CH_3$). Amide formation occurred even more readily with oxalic acid, and 2 : 4-*diamino-6-hydroxypyrimidyl-5-oxamic acid* (I; R = $NH{\cdot}CO{\cdot}CO_2H$) separated rapidly in high yield from hot aqueous solutions of the reactants. This oxamic acid was soluble in hot sodium hydrogen carbonate solution, and on cooling the sparingly soluble *sodium* salt was obtained. The oxamic acid is undoubtedly an intermediate in the usual preparation of leucopterin (III) by fusing the pyrimidine (I; R = NH_2) with oxalic acid dihydrate at 160—260° (Purrmann, *Annalen*, 1940, **544**, 188; Totter, *J. Biol. Chem.*, 1944, **154**, 105).

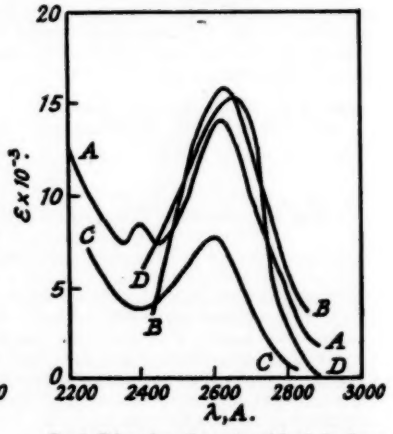
The facile condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine with aqueous oxalic acid suggested that the participating 5-amino-group might be abnormally reactive. Further work is necessary to establish this, since the insolubility of the product would tend to favour the reaction.

FIG. 1.



A. *Leucopterin in* 0·1N-*sodium hydroxide.*
B. 2 : 4-*Diamino-6-hydroxypyrimidyl-5-oxamic ocid in* 0·1N-*sodium hydroxide.*
C. *Thymine in water.*

FIG. 2.



2 : 4-*Diamino-5-acetamido-6-hydroxypyrimidine;* A, *in* 0·1N-*sodium hydroxide;* B, *in* 0·1N-*hydrochloric acid.*
2 : 4-*Diamino-5-α-hydroxypropionamido-6-hydroxypyrimidine;* C, *in* 0·1N-*sodium hydroxide;* D, *in* 0·1N-*hydrochloric acid.*

The lack of reactivity of amino-groups in positions 2 and 4 was illustrated by the recovery of 2 : 4-diamino-6-hydroxypyrimidine on attempted benzoylation by the Schotten–Baumann method.

The ultra-violet absorption characteristics of the above pyrimidines are summarised in the table. For these measurements the author is indebted to Dr. W. F. Elvidge and Mr. R. C. Voss of the Analytical Department, Boots Pure Drug Co. Ltd. Most of the acylamido-compounds have similar spectra, although prepared by different methods. This confirms the view that they are all 5-acylamides, but spectrographic data alone would be inconclusive without the examination of some 2- and 4-acylamides.

Heyroth and Loofbourow (*J. Amer. Chem. Soc.*, 1934, **56**, 1728) correlated the absorption intensities with the degree of unsaturation in the pyrimidine ring, which is changed by tautomerism when hydroxy- or amino-groups are in positions 2, 4, or 6. The intensity maxima (ϵ = 10,000—20,000) of the 2 : 4 : 5-triamino-6-hydroxypyrimidine derivatives are intermediate between that of barbituric acid and those of uracil and thymine. The structures (I*A*) or (I*B*) for the compounds are a convenient representation of the relative reactivities of the amino-groups and indicate a relationship to barbituric acid and uracil. Resonance probably plays an important part in such molecules, but this factor cannot be usefully discussed here.

Measurements have been carried out on some of the compounds in both acid and alkaline

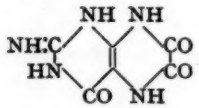
solutions (Fig. 2). The maxima are usually accentuated by acids, but, although this may be due to the tautomers (I*B*) being favoured, caution is essential in interpreting these results, since some of the compounds are unstable in alkaline solutions.

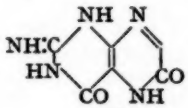
The absorption curve of thymine alters with the pH, but in neutral or acid solutions a curve is obtained, with $\lambda_{max.}$ 2640 A. ($\epsilon_{max.}$ 7800) (Fig. 1), resembling those obtained with the 5-acylamidopyrimidines. Under these conditions, thymine probably exists in the form (II; R = Me), which recalls structure (I*A*) for the 5-acylamidopyrimidines.

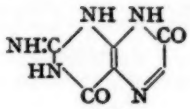
The 5-benzylideneamino- and 5-benzamido-compounds gave curves of a different type, attributed to the presence of the powerful phenyl chromophore. Although the sulphonamide has a maximum absorption at 2590 A., this is not believed to be significant since sulphanilamide also absorbs at this wave-length (Elvidge, *Q. J. Pharm.*, 1941, **14**, 141).

Leucopterin (III) and the oxamic acid (I; R = NH·CO·CO_2H) are physically similar, and differ chemically only by one molecule of water. The absorption curves are, however, quite distinct (Fig. 1).

The assignment of structures (IV) and (V) to xanthopterin and *iso*xanthopterin was originally based on the facile conversion of haloacetyl derivatives (assumed to be the 5-acylamides) of 2 : 4 : 5-triamino-6-hydroxypyrimidine into xanthopterin (Purrmann, *Annalen*, 1940, **546**, 98; Wellcome, B.P. 579,138). Gates (*Chem. Reviews*, 1947, **41**, 81) preferred a proof based on a complex series of degradations which have recently been carried out. The original proof was simpler, and, in the light of the present work, is adequate.


(III.)


(IV.)


(V.)

2 : 4 : 5-Triamino-6-hydroxypyrimidine was converted by a standard method into 2 : 4-*diamino*-5-p-*acetamidobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = NH·SO_2·C_6H_4·NHAc), which was then deacetylated to 2 : 4-*diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = NH·SO_2·C_6H_4·NH_2). The author is indebted to Mr. C. E. Coulthard of the Bacteriological Department, Boots Pure Drug Co. Ltd., for the results of a preliminary biological examination of this sulphanilamidopyrimidine. It was less active than sulphanilamide in inhibiting the growth of a number of bacteria, including *Streptococcus hæmolyticus*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Pseudomonas pyocyanea*.

Experimental.

(Temperatures are uncorrected. Compounds for which no m. p.s are given decomposed on heating.)

2 : 4 : 5-*Triamino*-6-*hydroxypyrimidine* (I; R = NH_2).—The intermediate 2 : 4-diamino-6-hydroxypyrimidine sulphate was most satisfactorily prepared by the method of Traube and Dudley (*Ber.*, 1913, **46**, 3843). The subsequent procedure for conversion into the sodium hydrogen sulphite compound of the triaminopyrimidine was similar to those described recently (Totter, Shukers, Kolson, Mims and Day, *J. Biol. Chem.*, 1944, **152**, 149; Cain, Mallette, and Taylor, *J. Amer. Chem. Soc.*, 1946, **68**, 1996).

2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine* (I; R = NHAc).—The sodium hydrogen sulphite compound of 2 : 4 : 5-triamino-6-hydroxypyrimidine (2·45 g.) in water (5 c.c.) was treated with 2N-sodium hydroxide (20 c.c.) and acetic anhydride (2·0 c.c.) at 5°. The product was precipitated by carbon dioxide and recrystallised from boiling water. 2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine* formed pale yellow crystals, m. p. 343—344° (decomp.) (Found : N, 38·2. $C_6H_9O_2N_5$ requires N, 38·2%). The compound was soluble in dilute acids and dilute alkalis.

Ethyl Acetyloximinocyanoacetate.—Ethyl oximinocyanoacetate (Conrad and Schulze, *Ber.*, 1909, **42**, 735) was acetylated, after Cerchez and Colesui (*Compt. rend.*, 1932, **194**, 1954). The acetyl compound had b. p. 120—128°/1 mm. (lit., b. p. 148°/14 mm.) and was eventually obtained solid. It formed white crystals, m. p. 44°, from light petroleum (Found : N, 15·4. Calc. for $C_7H_8O_4N_2$: N, 15·2%).

Ethyl Acetamidocyanoacetate.—Aluminium amalgam reduction of the above oxime acetate (Cerchez and Colesui, *loc. cit.*) was unsatisfactory and the following method was adopted. Ethyl oximinocyanoacetate (28 g.) was dissolved in glacial acetic acid (150 c.c.), and zinc dust (32 g.) added during 1 hour with vigorous stirring and cooling to 25—30°. Acetic anhydride (20 c.c.) and acetic acid (50 c.c.) were added, and the mixture stirred for 2 hours at 70—80°. The zinc acetate was filtered off and washed with glacial acetic acid (4 × 50 c.c.). The acetic acid was distilled off under reduced pressure and the residue triturated with ethyl acetate, giving the acetamido-ester (21·2 g., 64%), m. p. 103—106°. This material was pure enough for the following reaction, in spite of the low m. p. Recrystallisation from ethyl acetate gave the pure compound, m. p. 129°.

Independent Synthesis of 2 : 4-*Diamino*-5-*acetamido*-6-*hydroxypyrimidine.*—Guanidine hydrochloride (4·8 g.) was dissolved in a solution of sodium ethoxide, prepared from sodium (2·3 g.) and ethanol (50 c.c.). After 5 minutes, ethyl acetamidocyanoacetate (8·5 g.) was added and the mixture was refluxed for 1 hour. The alcohol was removed in a vacuum, the residue dissolved in water (50 c.c.), and carbon

dioxide passed in to precipitate the pyrimidine [4·8 g.; m. p. 339—341° (decomp.), not depressed on mixing with a specimen prepared by direct acetylation of 2 : 4 : 5-triamino-6-hydroxypyrimidine]. With picric acid in water, the *picrate* was obtained in yellow needles, m. p. 254—255° (decomp.) (Found : N, 26·95. $C_6H_9O_2N_5,C_6H_3O_7N_3$ requires N, 27·15%). The same picrate (m. p. and mixed m. p.) was obtained from the acetamido-compound prepared by direct acetylation.

2 : 4-*Diamino-5-benzamido-6-hydroxypyrimidine* (I; R = NHBz).—2 : 4 : 5-Triamino-6-hydroxypyrimidine sulphate (2·4 g.) was dissolved in N-sodium hydroxide (40 c.c.), and benzoyl chloride (1·6 g.) added with shaking and cooling. Saturation with carbon dioxide precipitated a solid (2·6 g.; m. p. 313°), which on recrystallisation from dilute acetic acid gave the pure 5-*benzamido*-compound as pale yellow needles, m. p. 318—319° (Found : N, 28·6. $C_{11}H_{11}O_2N_5$ requires N, 28·6%).

2 : 4-*Diamino-5-α-hydroxypropionamido-6-hydroxypyrimidine* [I; R = NH·CO·CH(OH)·CH_3].—The pyrimidine sodium hydrogen sulphite compound (4·9 g.) and DL-lactic acid (3·6 g.) were heated at 115—125° (bath) for 1 hour. The product was washed with water and crystallised from the same solvent, giving pale yellow crystals of 2 : 4-*diamino-5-hydroxypropionamido-6-hydroxypyrimidine*, m. p. 299—301° (decomp.) (Found : N, 32·65. $C_7H_{11}O_3N_5$ requires N, 32·85%). The substance was soluble in dilute acids and dilute alkalis, but did not appear to form a picrate.

2 : 4-*Diamino-6-hydroxypyrimidyl-5-oxamic acid* (I; R = NH·CO·CO_2H).—The sodium hydrogen sulphite compound (10·9 g.) and oxalic acid dihydrate (12·4 g.) were dissolved in water (2220 c.c.) and boiled for 3 hours. The *oxamic acid* (7·3 g.) was filtered off and washed with hot water (Found : C, 33·9; H, 3·3; N, 32·4. $C_6H_7O_4N_5$ requires C, 33·8; H, 3·3; N, 32·8%). It resembled leucopterin in appearance, solubility in 2N-sodium hydroxide, and insolubility in all organic solvents. Unlike leucopterin, it dissolved in hot 5N-sulphuric acid and, with effervescence, in hot 6% sodium hydrogen carbonate solution. On cooling the latter solution, the sparingly soluble *sodium* salt separated (Found : N, 30·45. $C_6H_6O_4N_5Na$ requires N, 29·8%). The sodium salt (3·0 g.) with N-hydrochloric acid (20·0 c.c.) regenerated the oxamic acid (2·4·6 g.; calc. 2·7·2 g.), and sodium chloride (7·8 g.; calc. 7·5 g.) was obtained on evaporation of the filtrate.

Compound.	Solvent.	$\lambda_{max.}$ (A.) ($\epsilon_{max.}$).	$\lambda_{min.}$ (A.) ($\epsilon_{min.}$).
2 : 4-Diamino-6-hydroxypyrimidine	0·1N-NaOH	2620—2660 (13,220)	—
	0·1N-HCl	2630 (19,200)	—
2 : 4-Diamino-6-hydroxypyrimidine sulphate (1)	0·1N-NaOH	2620 (22,050); 2450 (10,140)	2470 (9100); 2410 (9100)
2 : 4 : 5-Triamino-6-hydroxypyrimidine sulphate (2)	0·1N-NaOH	2400 (5230); 2650 (5450); 2740 (5700)	2380 (4990); 2520 (4250); 2670 (5230)
2 : 4-Diamino-5-acetamido-6-hydroxypyrimidine (Fig. 2)	0·1N-NaOH	2400 (8400); 2610 (14,020)	2350 (7470); 2450 (7470)
	0·1N-HCl	2630 (15,900)	—
2 : 4-Diamino-5-(α-hydroxypropionamido)-6-hydroxypyrimidine (Fig. 2)	0·1N-NaOH (3)	2600 (7900)	2380 (3860)
	0·1N-HCl	2650 (15,300)	—
2 : 4-Diamino-6-hydroxypyrimidyl-5-oxamic acid (Fig. 1)	0·1N-NaOH	2630 (11,500)	2450 (5300)
2 : 4-Diamino-5-benzylideneamino-6-hydroxypyrimidine (4)	Water	2360 (4250); 2850 (3100)	2660 (800)
2 : 4-Diamino-5-benzamido-6-hydroxypyrimidine	0·1N-NaOH	2280—2300 (23,300)	—
2 : 4-Diamino-5-(*p*-aminobenzenesulphonamido)-6-hydroxypyrimidine	0·1N-NaOH	2590 (22,800)	2250 (8600)
Leucopterin (5) (Fig. 1)	0·1N-NaOH	2400 (16,800); 2850 (6880); 3420 (9940)	2350 (13,800); 2710 (5590); 3050 (3960)
Thymine (6) (Fig. 1)	0·1N-NaOH	2700 (5500); 2780 (5450); 2800—2860 (5250) (inflexion)	2520 (2520); 2730 (5050)
	Dilute ammonia	2640 (7650)	—
	Water or 0·1N-HCl (7)	2640 (7800)	2420 (2000)

Notes.

(1) $(C_4H_6ON_4)_2,H_2SO_4$; *M*, 350.
(2) $C_4H_7ON_5,H_2SO_4$; *M*, 239. The compound decomposed quickly in alkaline solution. The measurements were made with a fresh solution. The curve obtained suggested that two similar compounds were present.
(3) This compound was unstable in alkaline solution; the measurements were therefore made on freshly prepared solutions.
(4) Prepared after Traube and Nithak (*Ber.*, 1906, **39**, 235).
(5) Prepared after Totter (*J. Biol. Chem.*, 1944, **154**, 105). The absorption figures agree with those recorded by Jacobsen and Simpson (*Biochem. J.*, 1946, **40**, 3).
(6) Prepared after Wheeler and McFarland (*Amer. Chem. J.*, 1910, **43**, 19).
(7) Deduced from the curve given by Stimson and Reuter (*J. Amer. Chem. Soc.*, 1945, **67**, 847).

Attempted Benzoylation of 2 : 4-*Diamino-6-hydroxypyridine.*—The diamine sulphate (3·7 g.) dissolved in 0·7N-sodium hydroxide (60 c.c.) was treated with benzoyl chloride (3·5 g.) and 2N-sodium hydroxide (30 c.c.) at 10°. After 2 hours, the solution was neutralised with 2N-hydrochloric acid, and benzoic acid (1·4 g.; m. p. 122°) was removed by filtration. Left at 0°, the mother liquors gave 2 : 4-diamino-6-hydroxypyrimidine (1·4 g.), bladed needles m. p. 277—279° (decomp.), raised to m. p. 286—288°

(decomp.) on crystallising from water (Found in material dried at 100°/1 mm.: N, 44·3. Calc. for $C_4H_6ON_4$: N, 44·4%) [Conrad, *Annalen*, 1905, **340**, 313, gives m. p. 286° (decomp.)].

2 : 4-*Diamino*-5-p-*acetamidobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NHAc$). —The triamino-pyrimidine sulphate (4·8 g.) was dissolved in 0·3N-sodium hydroxide (120 c.c.) and stirred at 60—70° whilst *p*-acetamidobenzenesulphonyl chloride (5·2 g.) and 2N-sodium hydroxide (10 c.c.) were added. The mixture was stirred at 80° for 30 minutes, and was then neutral. The solid (4·7 g.) was washed with water and dried in a vacuum. Recrystallisation from boiling water gave the 5-p-*acetamidobenzenesulphonamide* in small, pale yellow needles, frothing at 280° (Found: N, 24·4. $C_{12}H_{14}O_4N_6S$ requires N, 24·85%).

2 : 4-*Diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* (I; R = $NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NH_2$).—The above acetyl compound (1·8 g.) was boiled for 20 minutes with 5N-sodium hydroxide (20 c.c.). The solution was neutralised with hydrochloric acid, and the solid (1·1 g.) recrystallised from boiling water. 2 : 4-*Diamino*-5-p-*aminobenzenesulphonamido*-6-*hydroxypyrimidine* formed small, pale brown crystals (Found: N, 28·15. $C_{10}H_{12}O_3N_6S$ requires N, 28·4%). The compound was much more soluble in water than the acetyl derivative. During the preparation, only a trace of ammonia was evolved. Hydrolysis of the acetyl compound with boiling 2N-hydrochloric acid gave a non-basic white compound, which was not the required sulphonamide.

The author thanks Drs. W. F. Short and D. A. Peak for their interest in this work. He is also indebted to Mr. C. A. Bartram for experimental assistance.

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230. *A Thermochemical Evaluation of the Bond Strengths in Some Carbon Compounds. Part I. (Methyl Carbon)–Iodine and (Methyl Carbon)–Magnesium.*

By H. MACKLE and A. R. UBBELOHDE.

Thermochemical schemes for the evaluation of the bond strengths of carbon–iodine in organic iodides and of carbon–magnesium in Grignard compounds have been devised and tested. The heats of the reactions involved have been determined where necessary in a specially constructed calorimeter. From these data it is calculated that the heat of dissociation $D(CH_3{-}I) = 54{\cdot}7 \pm 1{\cdot}4$ kcals. The agreement with the value (54 kcals.) obtained from kinetic data substantiates a mechanism previously proposed for pyrolysis of methyl iodide.

A rather less accurate value is estimated from the experimental data for $D(CH_3{-}Mg) = 67{\cdot}0$ kcals.

In testing the thermochemical schemes used, the conditions governing the relative extents of the competing reactions in ethereal solution, *viz.*, $CH_3{\cdot}MgI + I_2 \longrightarrow CH_3I + MgI_2$ and $2CH_3{\cdot}MgI + I_2 \longrightarrow C_2H_6 + 2MgI_2$, have been investigated experimentally with special reference to the possible role of the equilibrium $2CH_3{\cdot}MgI \rightleftharpoons Mg(CH_3)_2 + MgI_2$. The concurrent attack of free methyl radicals on the ether has also been examined.

ACCURATE values of the heats of dissociation of various chemical bonds are still very limited in number. Particularly for organic compounds, the great body of information on chemical reactivity completely swamps the information on chemical bond strength. When reactivity can be expressed in terms of quantitative kinetics, the activation energies obtained (Rice and Johnson, *J. Amer. Chem. Soc.*, 1934, **56**, 214; Butler and Polanyi, *Trans. Faraday Soc.*, 1943, **39**, 19) may throw some light on the chemical binding in the ground states of the molecules participating in the reaction. But in general, organic chemistry is the chemistry of activated states of molecules. Theories relating bond structure with chemical and thermodynamic behaviour can only be tested properly by quantitative data on the ground states. Accurate data of this kind are particularly important for assessing departures from the additivity rule for bond energies arising because of resonance, steric factors, etc.

Within recent years, valuable advances have been made in the more accurate evaluation of bond strengths of organic compounds, for example in homologous series, by combustion calorimetry (Rossini, *Bull. Bur. Stand. J. Res.*, 1934, **13**, 29, 189; 1939, **22**, 115). Differences in the heats of formation of related hydrocarbons have also been determined from hydrogenation reactions (Kistiakowsky, *J. Amer. Chem. Soc.*, 1936, **58**, 137; 1937, **59**, 831; *J. Chem. Physics*, 1942, **10**, 305). But in the case of the more reactive or labile organic compounds where bond strengths are of particular interest, combustion calorimetry may be handicapped by technical difficulties.

The present paper describes a method of obviating combustion calorimetry by using some of the reactions of Grignard reagents for the purposes of evaluating heats of reaction, and hence of obtaining bond energies in certain covalent organic compounds. The scope of the experiments

was limited in the first instance to the evaluation of carbon–halogen bonds, though the methods have obvious possibilities of extension in view of the wide range of organometallic compounds now known (Gilman, " Advanced Treatise on Organic Chemistry ", Wiley, 1943). The data obtained can also be used to evaluate, rather less accurately, the carbon–magnesium bond strength in the Grignard compound used.

The selection of the best experimental conditions involves two main problems : (*a*) selection and control of the chemical reactions whose heat values are to be evaluated; (*b*) selection of the calorimetric techniques.

Preliminary experiments, and records in the literature, made it clear that the problem of controlling Grignard reactions so as to give consistent and useful heat data might involve many difficulties. On the other hand, fairly simple calorimetric techniques were found to give reliable heat data, at any rate to the accuracy with which chemical control could be achieved. A brief discussion of the chemical reactions may serve to illustrate the kind of difficulties encountered.

Each of the chemical reactions used must be amenable to accurate quantitative evaluation, and must preferably proceed to completion. Any competing reactions must be susceptible to accurate evaluation and control.

The most obvious reaction to select for thermochemical investigation is the direct formation of the Grignard reagent from metallic magnesium and methyl iodide :

$$CH_3I + Mg + Et_2O \longrightarrow CH_3{\cdot}MgI\,(Et_2O) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In fact, preliminary experiments showed that this reaction was not very convenient. Difficulties encountered included the variable induction period, which interferes with the timing required for calorimetric corrections. Again, side reactions such as the Wurtz reaction

$$2CH_3I + Mg + (Et_2O) \longrightarrow C_2H_6 + MgI_2\,(Et_2O) \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

and possibly also attack of the solvent by free methyl radicals (see below)

$$2CH_3I + Mg \longrightarrow MgI_2 + 2CH_3\text{-} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

led to the loss of methyl iodide in processes competing with (1). The amount of these side reactions could be determined, *e.g.*, by analysing the gases formed, but the experimental evaluations were awkward in assessing the thermochemical corrections. Furthermore, the formation of the Grignard compound does not necessarily proceed to completion whether excess of magnesium or of methyl iodide is used, and this again may involve undesirably large correction terms.

Again, equation (1) calls for the use of very pure methyl iodide, free from iodine, water and other impurities, if reliable thermochemical data are to be obtained. The purification of this substance to the requisite very high degree is not very convenient when a large number of experiments have to be carried out.

The possibility was next investigated of starting with the Grignard compound pre-formed in the calorimeter, and of measuring the heat evolved on decomposing this compound in various ways. Decomposition by water, added either pure or in ethereal solution, involves the reaction

$$CH_3{\cdot}MgI + H_2O \longrightarrow CH_4 + \tfrac{1}{2}MgI_2 + \tfrac{1}{2}Mg(OH)_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Calorimetrically, this reaction is not very suitable, owing to the fact that magnesium hydroxide (or hydroxyiodide) has a variable and rather large lattice energy. A further complication is that the volume of gas evolved is rather large and calls for special experimental treatment.

Investigation showed that decomposition of methylmagnesium iodide by iodine in ethereal solution involved the reactions

$$CH_3{\cdot}MgI + I_2 \longrightarrow CH_3I + MgI_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$
(from 90% to 99·7% under the conditions detailed below)

$$2CH_3{\cdot}MgI + I_2 \longrightarrow C_2H_6 + MgI_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$
(from 10% to 0%)

together with attack of the solvent by methyl

$$(2CH_3\text{-}) + (C_2H_5)_2O \longrightarrow 2CH_4 + CH_3{\cdot}CHO + C_2H_5 \quad . \quad . \quad . \quad . \quad . \quad (7)$$
(from about 0·25% to 0%)

It was originally planned to use reaction (5) together with the reaction

$$CH_3{\cdot}MgI + HI \longrightarrow CH_4 + MgI_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

to evaluate the carbon–iodine bond strength from known dissociation energies. However, it

was found that the course of (5) and (6) could be controlled with such precision that it was possible to evaluate the individual heats of reaction

$$\underset{(Et_2O)}{CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} \longrightarrow \underset{(Et_2O)}{CH_3I} + \underset{(Et_2O)}{MgI_2} \quad \text{(A)}$$

and

$$\underset{(Et_2O)}{2CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} \longrightarrow \underset{(gas)}{C_2H_6} + \underset{(Et_2O)}{2MgI_2} \quad \text{(B)}$$

without having recourse to reaction (8). Precision of reproducibility was verified both from measurements of the heats evolved, and by means of detailed analysis of the reaction products. The principal factors involved in the control of reactions (5) and (6) are the overall concentration, the concentration of magnesium iodide, and the temperature.

From (A) and (B) it could be calculated, as discussed in detail below, that

$$\underset{(gas)}{CH_3I} \longrightarrow \underset{(gas)}{CH_3} + \underset{(gas)}{I} + 54{\cdot}75 \pm 1{\cdot}43 \text{ kcals.}$$

and

$$\underset{(gas)}{CH_3{\cdot}MgI} \longrightarrow \underset{(gas)}{CH_3} + \underset{(gas)}{-MgI} + 67{\cdot}00 \text{ kcals.}$$

It may be added that the scheme of reactions (5)—(8) would appear to offer further advantages over, say, (3) and (4) in extending the present methods to other Grignard compounds. The difficulties in initiating the formation of Grignard compounds in some cases is well known, and this seriously handicaps the use of (3) for thermochemical work, whereas once the compounds have been formed (5) and (6) usually proceed smoothly.

Calorimetric Techniques.—The calorimeter was deliberately kept simple (see below) in order to facilitate investigation and control of the competing chemical reactions. In essence, the reactions used involved the mixing of ethereal solutions of the Grignard compound with ethereal solutions of iodine. In order to facilitate the carrying out of a large number of determinations, a special mixing pipette was used, described below.

The temperature rise resulting on mixing, measured in arbitrary units of galvanometer deflection, was matched as accurately as possible by adjusting a measured input of electrical energy so as to give as nearly as possible the same temperature rise and rate of temperature rise under the same conditions (cf. Rossini, *Chem. Reviews*, **1936**, **18**, 235; Eucken, " Handbuch der experimental Physik ", VIII, Teil 1, pp. 48 *et seq.*).

Experimental.

Preparation of the Solution of Grignard Compound.—Fig. 1 shows the apparatus used; *A* is a " Pyrex " vessel of about 100 c.c. capacity; the upper end of the condenser was protected from moisture and carbon dioxide by layers of calcium chloride and soda-lime, and normally a slow stream of dry oxygen-free nitrogen flowed through it.

Specially pure dry crystalline magnesium (Fe, 0·0004; Zn, 0·003; Pb, 0·002; Si, Ni, Sn, Cd, 0·005%), obtained by courtesy of Magnesium Metal Corporation, was broken into small pieces in a warm mortar and dried in a vacuum oven at 60° before use. About 2 g. were inserted, and covered with pure ether. The required amount of redistilled methyl iodide was then added, and the solution refluxed till analysis in test experiments showed that the reaction would proceed no further (about 30 mins.). Traces of magnesium hydroxide in the solution were allowed to settle, a small flow of pure, dry, oxygen-free nitrogen being maintained to prevent back diffusion of moisture. Amounts of this clear solution were siphoned over as required by replacing the stopper *B* by a stopper *C*, and closing the vent tube *E* with a finger. Before closing this vent, the receiving vessel *D* was swept out with dry, oxygen-free nitrogen. A small counter current of this nitrogen was maintained throughout as shown, to prevent back diffusion of moisture. This completely obviated the loss of any reagent by hydrolysis. 10-C.c. portions of the reagent could be pipetted from *D* either into the calorimeter (previously swept out with nitrogen) or into water for analysis, in each case the tip of pipette being protected continuously by nitrogen and the mouthpiece fitted with a calcium chloride–soda-lime tube.

Pure dry nitrogen was prepared by passing cylinder nitrogen over copper gauze washed with ammonia (*d* 0·880) to remove oxygen, and then washing the gas with concentrated sulphuric acid, potassium hydroxide pellets, and a phosphoric oxide tube to remove the last traces of water vapour. All joints were sealed with a beeswax–rosin mixture (1 : 1).

Preparation of Pure Dry Ether.—Great care was exercised in the preparation of the ethyl ether, since possible catalytic effects from impurities might affect the relative importance of competing reactions in the calorimeter. The steps described below were modified where convenient from previously published methods. Portions of 1000 c.c. of commercial ether were shaken with the following: (1) ten times with 50-c.c. portions of distilled water; (2) once with a solution containing 4·5 parts of saturated potassium hydrogen carbonate solution and 1 part of saturated mercuric chloride solution (Poleck and Thummel, *Ber.*, **1889**, **22**, 2863) (after this stage the ether should not give the iodoform test for alcohols); (3) strongly alkaline potassium permanganate solution in 50-c.c. portions till no further reduction occurred (to remove aldehydes); (4) a concentrated ferrous sulphate solution to remove any remaining peroxides; (5) finally, with more water. The ether was then allowed to stand overnight with calcium chloride, and was bottled

in Winchester quart bottles with sodium wire for at least a week, before distillation. (6) A 2-l. still was filled from time to time with ether treated as above, (1)—(5). The distillation was carried out in an atmosphere of oxygen-free nitrogen. The ether vapour was allowed to pass over sodium hydroxide pellets (Hammond, U.S.P. 1923, 1466435), thence by way of a condenser into a receiver, from which it could be pipetted off as required by using a counter current of dry, oxygen-free nitrogen during any transfer. All pipettes were protected at the mouthpiece with a tap and a soda-lime tube.

The improvement in purity of the ether, compared with ether merely dried over sodium wire, was verified by the substantial shortening of the induction period in preparing methyl- and ethyl-magnesium iodides and by a somewhat increased yield (Mackle, in the press).

Ether thus prepared was a rather costly solvent (final yield 25% of initial quantity) and the residues after calorimetry, etc., which had never been exposed to oxygen, were fractionally distilled again to recover the pure ether. Induction period measurements (Mackle, *loc. cit.*) verified that no loss of purity had occurred during the reactions involved.

Calorimetry.—After some preliminary designs had been tested, the final design of calorimeter used was as shown in Fig. 2. It consists essentially of a copper vessel (*A*) with paraffin (b. p. 120—150°) as

FIG. 1.

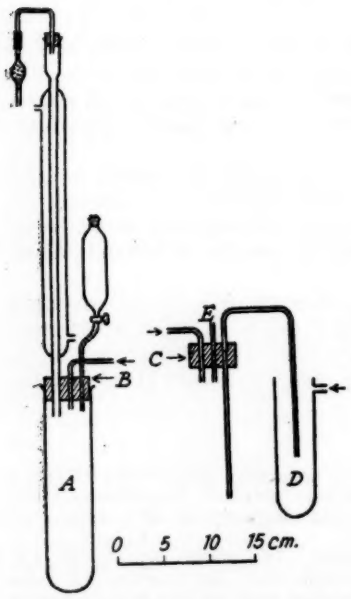
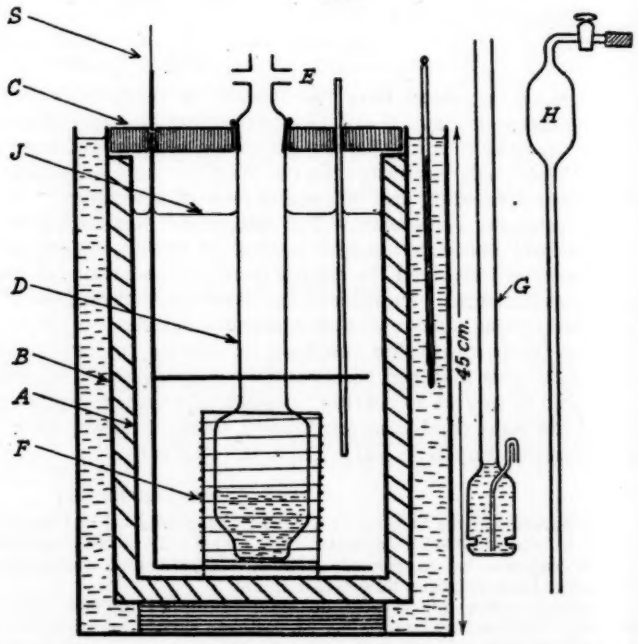


FIG. 2.



calorimeter fluid. (The paraffin oil level is shown at *J*.) This vessel is insulated from a water-bath "environment" whose temperature is adjustable to be as nearly as possible the same as that of the calorimeter. Thermal insulation is obtained by means of a layer $\frac{1}{4}$" thick of *magnesia alba levis* carefully dried and sealed from the air, and contained in an outer metal jacket (*B*). A lid of perspex (*C*) is coated on the under side with aluminium foil, to minimise heat exchange by radiation. The reciprocating mechanical stirrer *S* is fitted with vanes (not shown). The lower part of the stirrer is made of copper, and the part projecing from the calorimeter of glass tubing, to minimise heat transfer.

Temperature changes of the calorimeter fluid were measured by means of a 5-junction copper-constantan thermocouple with a galvanometer and lamp and scale (0·02° = 1 mm. approx.). The temperature scale was calibrated against a standard N.P.L. thermometer, in order to obtain the approximate temperature of reaction, though estimates of heats of reaction do not depend on this calibration (see below).

An electric heating coil was constructed by winding bare "Ferry" nickel–chrome wire of negligible temperature coefficient, non-inductively on a "Pyrex" frame. This frame fits closely over the reaction vessel as indicated by *F* so as to facilitate "matching" the heat input into the calorimeter by the chemical and electrical processes.

The resistance (66·588 ± 0·020 ohms) of the heating coil was measured from time to time by means of a Carey-Foster arrangement. When the current through this coil was switched on, it was measured at intervals by determining the potential drop across a calibrated resistance of about 2 ohms.

The chemical reaction vessel is indicated by *D*. It consists of a "Pyrex" bulb, plated with copper on the outside up to the level of the frame of the heating coil, and fitted with a long neck. A small continuous flow of oxygen-free dry nitrogen is maintained in the upper part, at the joint (*E*), throughout the experiments, to prevent back diffusion of moisture. Normally the reaction vessel contains the solution of Grignard reagent. Special tests verified that the loss of ether vapour under these conditions was negligible.

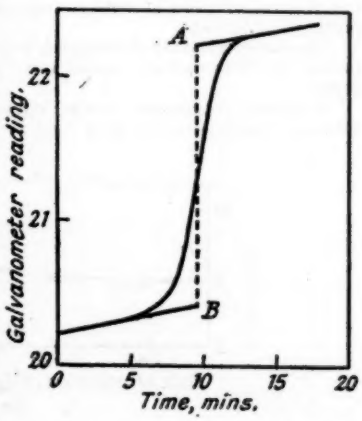
A special reaction pipette *G* (shown separately in Fig. 2) rests on the bottom of this reaction vessel; the pipette is closed at the top by means of an easy-fitting rubber sleeve (not shown). It is refilled *in situ* as required up to a calibration mark (*e.g.*, with a solution of iodine in ether, approx. 150 g./l.) whilst it is withdrawn up to the neck of the reaction vessel. For this filling, the long thin pipette *H* is used. Careful checks showed that the delivery of fluid from the reaction pipette could be reproduced to ± 0·25%.

In determining the heat evolved in a chemical reaction, the calorimeter was first allowed to come to approximate equilibrium, with the reaction vessel and pipette both filled. Temperature–time observations were then recorded, and at a convenient moment the contents of the pipette were discharged into the reaction vessel, by applying a measured pressure of nitrogen for the requisite interval of time, previously tested in blank experiments.

The contents of the reaction vessel were than gently stirred by raising and lowering the pipette several times through a few cm., so as to utilise the groove at the bottom, and the temperature–time observations were continued till a steady state was again well established.

From the temperature–time plot, the usual corrections were applied (cf. Roth, described in Eucken, *op. cit.*). The temperature of the external water-bath was adjusted so that the correction did not exceed 5% of the actual change in temperature due to chemical reaction, in the least favourable case, and 3·5% on the average. Furthermore, the calorimeter was calibrated from time to time by means of the electrical heating coil, with a heat input whose magnitude and duration were adjusted so as to give very nearly the same temperature–time curve as that obtained for the chemical reaction. Thus the change in thermo-e.m.f. reading due to the heat of reaction (corrected for any heat exchange) was equated directly to the electrical energy required to give a similar change in thermo-e.m.f., without depending, except in a subsidiary degree, upon the actual temperature scale used, or the precision with which the corrections for heat exchange with the surroundings could be calculated. Fig. 3 illustrates a typical temperature–time curve, from which the "true rise" was obtained by the standard procedure. Using convenient arbitrary units, the corrected mean rise for ten experiments with 100% reaction (5) was 70 ± 1 units, and for ten experiments with conditions chosen to give 90% reaction (5) and 10% reaction (6) the mean rise was 79 ± 1 units.

Fig. 3.



Electrical calibration under exactly the same conditions, with Grignard reagent in the reaction vessel and pure dry ether in the delivery pipette, gave as the mean of ten experiments with closely matched preliminary and post-heating curves 152·2 ± 0·2 cals. ≡ 78 ± 1 units.

Control of the Competing Reactions.—The major difficulty in carrying out the calorimetry of Grignard reactions was to ensure reproducibility of the extent to which the competing reactions (5)—(7) took place within the calorimeter. Special investigations were carried out to examine the influence of the more important variables on the yield of various products, as follows:

(i) For analysing the amount of methylmagnesium iodide present in the solution at any stage, the acid–alkali titration due to Gilman (*J. Amer. Chem. Soc.*, 1923, **45**, 150) was followed. Phenolphthalein was used as indicator. For one or two special experiments, the amount of methylmagnesium iodide in solution was evaluated from the volume of methane obtainable, following a method originally due to Zerewitinoff and studied in detail by Gilman (*ibid.*).

(ii) The amount of iodine in the solution delivered by the special reaction pipette was determined by test deliveries into excess of standard 0·1N-sodium thiosulphate. These were reproducible to ± 0·25%. Originally, iodine in hexane was used, as it was thought that this would be less susceptible to concentration changes due to loss of solvent, than iodine in ether. But with experience there was no difficulty in delivering reproducible quantities of iodine in ether, which are preferable on account of the higher concentrations, and because their use avoids changing the composition of the solvent obtained as a result of mixing.

(iii) When a solution of iodine in ether is added to the Grignard reagent, the extent to which the competing reactions take place depends somewhat on the temperature, the rate of mixing, and the concentration of the Grignard reagent.

The temperature of the solutions in the reaction vessel had to be kept at about 15° throughout the experiments, to avoid possible difficulties from vapour-pressure variations of the ether, so that this variable did not in fact alter in the present experiments. Theoretical considerations suggest that a substantial change in temperature might alter the relative importance of the reactions. For example, a substantial rise in temperature might increase the attack on the solvent ether by the free radicals formed (see below), by analogy with the photochemical observations of Norrish and Bamford (*J.*, 1938, 1544).

Experiments were carried out on the rate of mixing, since the relative extent of reactions (5) and (6) [cf. Fig. 4(*a*)] might be quite sensitive to such a factor. For instance, the fastest interaction between iodine and the Grignard reagent might occur in two steps:

$$(a)\ I_2 \rightleftharpoons 2I + 36\ \text{kcals.};\qquad (b)\ I + CH_3{\cdot}MgI = MgI_2 + CH_3^{-}\ \text{(approx. thermo-neutral)}$$

At the momentary boundary between a solution with excess of iodine and a solution with excess of methylmagnesium iodide the methyl radicals formed by the reaction $CH_3{\cdot}MgI + I \longrightarrow MgI_2 + CH_3$· have a momentary free existence before they either (*a*) combine with further iodine atoms or molecules to form methyl iodide, $CH_3 + I_2 \longrightarrow CH_3I + I$, or (*b*) combine with neighbouring hydrocarbon groups.

These might be other methyl radicals, which could form ethane or disproportionate [cf. reaction (6)], or to a limited extent the ethyl groups in the ether might be attacked [cf. reaction (7)].

On this supposition vigorous mixing would promote reaction (5) by ensuring that the methyl radicals have every chance to collide with iodine, whereas slow addition and a high concentration of Grignard reagent would promote reaction (6) by favouring further collisions with methyl or ethyl.

Experiments in varying the rate of addition from the reaction pipette were carried out in the reaction vessel. The extent to which the competing reactions occurred was determined by analysis of the ratio of methyl iodide formed to the iodine added.

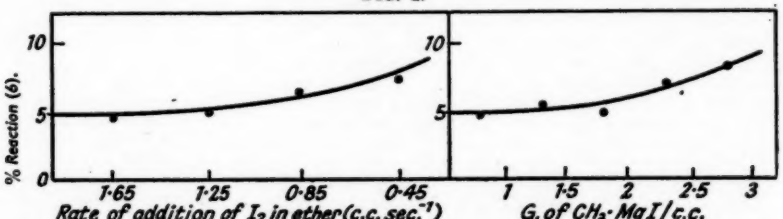
20 C.c. of the solution of methylmagnesium iodide, prepared as described above, were pipetted into the reaction vessel. The concentration of this iodide was determined separately on three samples of 10 c.c. each, by Gilman's method. A known amount of iodine, insufficient to complete the reactions (5) and (6), was added by means of the delivery pipette. The methylmagnesium iodide not used was then estimated as before. If only reactions (5) and (6) take place, the extent to which each occurs can be calculated from the iodine used, and the methylmagnesium iodide used, by simple proportion.

As a check on this calculation, the amount of methyl iodide formed in the reaction vessel was estimated, after hydrolysing the contents, by warming to 45° and bubbling oxygen-free nitrogen through it, and passing the vapours into alcoholic silver nitrate solution. From the weight of silver iodide precipitated, the weight of methyl iodide formed was calculated. Blank experiments carried out on the original solution of methylmagnesium iodide before adding iodine showed that no methyl iodide was present at the start.

These experiments distinguish between iodine used to form methyl iodide, as in (5), and iodine used to liberate hydrocarbon, by reactions (6) and (7). The further evaluation of the extent of (7) is discussed below.

A typical experiment was as follows: 2 G. of pure distilled magnesium, 50 c.c. of purified ether, and 5·0 c.c. of methyl iodide were used to prepare the solution of methylmagnesium iodide. Titration of the

FIG. 4.



original solution gave 1·738 ± 0·002 g. of methylmagnesium iodide per 20 c.c. Two successive additions of iodine solution were made from the delivery pipette *A* (total, 1·526 g. of iodine). Titration showed that the resulting solution contained 0·682 g. of Grignard reagent in 20 c.c., *i.e.*, the iodine used 1·056 g. of methylmagnesium iodide. But 1·526 g. of iodine would use 1·000 g. of methylmagnesium iodide if all decomposed according to reaction (5), and 2·000 g. if all decomposed according to reaction (6) or (7). Hence, in this case 94·5% of reaction (5) and 5·5% of reaction (6) or (7) had occurred.

In this particular experiment, the recovery of methyl iodide in the check determination, from the weight of silver iodide collected, corresponded with 94·0% of reaction (5). Similar checks were carried out a number of times to verify the assumptions made above.

Results plotted in Fig. 4(*a*) made it easy to select a rate of addition such that the course of the reaction was substantially independent of small accidental variations in the rate of mixing, and reproducible calorimetry could thus be ensured.

Finally, the concentrations of the various components before mixing might be expected to influence the relative extent of the competing reactions, for similar reasons to those discussed with reference to the rate of addition.

Fig. 4(*b*) gives the result of experiments to explore the effect of this variable. These were carried out under the same conditions as were used for calorimetry, except that no temperature–time curves were plotted. It will be noted that, even at the lowest concentration of methylmagnesium iodide used, reaction (6) occurred to the extent of 5%.

A useful discovery facilitated the obtaining of reactions particularly suitable for the calculations of bond strengths. If the views of Schlenk and Schlenk (*Ber.*, 1931, **64**, 734; cf. Noller, *Ber.*, 1929, **62**, 920; Evans, *J. Amer. Chem. Soc.*, 1934, **56**, 654) are accepted, then it should be possible to displace the equilibrium $2R \cdot MgI \rightleftharpoons MgR_2 + MgI_2$ to the left by adding excess of magnesium iodide. From the literature, the equilibrium constants appear to be of the order unity in most cases. It seemed highly likely that the reaction $MgR_2 + I_2 \longrightarrow MgI_2 + (2R)$ would lead to less formation of RI, and more formation of R_2 and disproportionation, than *e.g.*, $R \cdot MgI + I \longrightarrow MgI_2 + R$. This possibility was tested by adding 1 g. of anhydrous magnesium iodide for every 20 c.c. of the clear solution of Grignard reagent in the reaction vessels, before pipetting over the iodine. It was found that this completely suppressed the reaction (6) in favour of reaction (5). Consequently, by carrying out a series of calorimetric determinations first with methylmagnesium iodide as prepared directly and then with excess of magnesium iodide present, it was possible to obtain the heats of reaction for the processes

$$CH_3 \cdot MgI + I_2 \longrightarrow CH_3I + MgI_2$$

and

$$\left.\begin{aligned}(1-x)CH_3MgI + (1-x)I_2 &\longrightarrow (1-x)CH_3I + (1-x)MgI_2\\ xCH_3MgI + \tfrac{1}{2}xI_2 &\longrightarrow \tfrac{1}{2}xC_2H_6 + xMgI_2\end{aligned}\right\}$$

where x was determined by accurate analysis after the heat evolution was completed, and ranged between 10% and 0%. By this means, the values

$$\underset{(Et_2O)}{CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} \longrightarrow \underset{(Et_2O)}{CH_3I} + \underset{(Et_2O)}{MgI_2} - 45{\cdot}4 \pm 1 \text{ kcals. per mole}$$

and

$$\underset{(Et_2O)}{2CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} \longrightarrow \underset{(gas)}{C_2H_6} + \underset{(Et_2O)}{MgI_2} - 103{\cdot}4 \pm 3 \text{ kcals. per mole}$$

could be evaluated. The acquisitive convention is used for signs, *i.e.*, heat evolved is accounted lost by the system and is given a negative sign.

In describing the above experiments, no mention has yet been made of the allowance for the possibility of reaction (7) competing with reaction (6). Attack of solvent ether by free methyl radicals is known to occur under certain conditions (*Ann. Reports*, 1944, **41**, 184). The products include methane, ethane, ethylene, and acetaldehyde, as can be represented by rewriting reactions (6) and (7) as follows, starting with two methyl radicals in each case :

$$2CH_3\cdot \longrightarrow C_2H_6 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6a)$$

$$2CH_3\cdot + 2CH_3{\cdot}CH_2{\cdot}OEt \longrightarrow 2CH_4 + 2CH_3{\cdot}\dot{C}H{\cdot}OEt$$

$$\downarrow$$

$$2CH_3{\cdot}CHO + 2\dot{C}_2H_5$$

$$\downarrow$$

$$C_2H_4 + C_2H_6 \quad . \quad . \quad . \quad . \quad (7a)$$

Hence, if (7*a*) occurs to any extent, at the expense of (6*a*) it will lead to a substantially greater volume of gas, which will include ethylene.

To obtain experimental evidence on this point, a series of determinations were carried out on the gas evolved on adding a solution of iodine in ether to ethereal methylmagnesium iodide. In a typical case, 4 c.c. of iodine–ether added to excess of methylmagnesium iodide gave 6·2 c.c. of gas (corrected for the partial pressure of ether vapour) at 15°. Under these conditions, the extent of reaction (5) was known to be 90·2%. If all the remaining iodine was used according to reaction (6), 5·7 c.c. of ethane would be produced at 15°. The somewhat larger volume obtained implies that reaction (7) occurs to the extent of not more than 2·5% of the allowance originally made for reaction (6), *i.e.*, to not more than 0·25% of the overall process.

As a cross-check, the amount of ethylene in the gases was determined by gas-analysis using absorption by bromine water. The volume of ethylene in the 6·2 c.c. was 0·16 c.c., which again implies that under these conditions solvent attack occurs to the extent of not more than 0·25% of the overall process. The very small correction this involves in the evaluation of the calorimetric data is discussed below.

Discussion.

Using the experimental values for heats of reaction in ethereal solution, it is necessary to evaluate the heats of reaction for the same reactions carried out in the gas phase, in order to compute the bond energies.

If we formulate the cyclic process

$$\begin{array}{ccccc} \underset{(Et_2O)}{CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} & \xrightarrow{-45{\cdot}4} & \underset{(Et_2O)}{CH_3I} + \underset{(Et_2O)}{MgI_2} & \quad . \quad . \quad . \quad . \quad . \quad . \quad (9{\cdot}0) \\ \downarrow q_1 \quad \downarrow q_2 & & \uparrow q_3 \quad \uparrow q_4 & \\ \underset{(gas)}{CH_3{\cdot}MgI} + \underset{(gas)}{I_2} & \xrightarrow{Q_A} & \underset{(gas)}{CH_3I} + \underset{(gas)}{MgI_2} & \quad . \quad . \quad . \quad . \quad . \quad . \quad (9{\cdot}1) \end{array}$$

where q_1, q_2, q_3, q_4 are the increases in heat content for the processes of solution, in the sense indicated, then

$$-45{\cdot}4 = (q_1 + q_2 + q_3 + q_4 + Q_A \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9{\cdot}2)$$

Similarly for the cyclic process

$$\begin{array}{ccccc} \underset{(Et_2O)}{2CH_3{\cdot}MgI} + \underset{(Et_2O)}{I_2} & \xrightarrow{-103{\cdot}4} & \underset{(gas + Et_2O \text{ vapours})}{C_2H_6} + \underset{(Et_2O)}{2MgI_2} & \quad . \quad . \quad . \quad (10{\cdot}0) \\ \downarrow 2q_1 \quad \downarrow q_2 & & \uparrow q_3 \quad \uparrow 2q_4 & \\ \underset{(gas)}{2CH_3{\cdot}MgI} + \underset{(gas)}{I_2} & \xrightarrow{Q_B} & \underset{(gas)}{C_2H_6} + \underset{(gas)}{2MgI_2} & \quad . \quad . \quad . \quad (10{\cdot}1) \end{array}$$

$$-103{\cdot}4 = (2q_1 + q_2 + 2q_4 + q_5) + Q_B \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10{\cdot}2)$$

The term q_5 is inserted owing to the fact that in the calorimetric evaluation as carried out above the gas evolved is saturated with ether vapour. It is evaluated as below, from the heat of condensation of ether; *i.e.*,

$$\underset{(gas)}{C_2H_6} + \underset{(gas)}{x\text{Et}_2\text{O}} \longrightarrow \underset{(gas)}{C_2H_6} + \underset{(liq.)}{x\text{Et}_2\text{O}} - q_5$$

In separating the thermochemical components of equation (9·1) we may begin by writing, for the break-up of CH_3MgI and MgI_2 into radicals and atoms:

$$\underset{(gas)}{CH_3\cdot MgI} \longrightarrow \underset{(gas)}{CH_3} + \underset{(gas)}{Mg} + \underset{(gas)}{I} + Q_G$$

and

$$\underset{(gas)}{MgI_2} \longrightarrow \underset{(gas)}{Mg} + \underset{(gas)}{2I} + Q_M$$

Equation (9·1) is equivalent to

$$Q_G + D(\text{I–I}) - D(\text{CH}_3\text{–I}) - Q_M = Q_A \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9{\cdot}3)$$

By applying the same procedure to equation (10·1) we can write

$$2Q_G + D(\text{I–I}) - D(\text{CH}_3\text{–CH}_3) - 2Q_M = Q_B \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10{\cdot}3)$$

i.e.

$$\tfrac{1}{2}Q_B = Q_G - Q_M + \tfrac{1}{2}D(\text{I–I}) - \tfrac{1}{2}D(\text{CH}_3\text{–CH}_3) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10{\cdot}4)$$

Subtracting (10·4) from (9·3), we have

$$Q_A - \tfrac{1}{2}Q_B = \tfrac{1}{2}D(\text{I–I}) + \tfrac{1}{2}D(\text{CH}_3\text{–CH}_3) - D(\text{CH}_3\text{–I}) \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

or, inserting the experimental values from (9·2) and (10·2) and rearranging:

$$\begin{aligned} D(\text{CH}_3\text{–I}) &= \tfrac{1}{2}D(\text{I–I}) + \tfrac{1}{2}D(\text{CH}_3\text{–CH}_3) + (\tfrac{1}{2}Q_B - Q_A) \\ &= \tfrac{1}{2}D(\text{I–I}) + \tfrac{1}{2}D(\text{CH}_3\text{–CH}_3) - 6{\cdot}3 \pm 1 \\ &\quad + (\tfrac{1}{2}q_2 + q_3 - \tfrac{1}{2}q_5) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12) \end{aligned}$$

It will be clear that with this particular combination of thermochemical terms, many of the corrections cancel or combine in such a way as to minimise the errors. Furthermore, inspection of equation (19) (below) shows that the net contribution from the present experimental data is small in the scheme utilised, so that the influence of the larger errors is minimised. The terms in equation (12) are evaluated as follows:

$$\tfrac{1}{2}D(\text{I–I}) = 18{\cdot}20 \pm 0{\cdot}03 \text{ kcals.} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

(Brown, *Physical Rev.*, **1931**, **38**, 709).

$$\tfrac{1}{2}D(\text{CH}_3\text{–CH}_3) = 42{\cdot}15 \pm 1{\cdot}1 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

(Rossini, *Bull. Bur. Stand. J. Res.*, **1934**, **13**, 28, 189; Kistiakowsky, *J. Chem. Physics*, 1942, **10**, 305; Skinner, *Nature*, **1946**, **158**, 592; U.S. Dept. of Commerce, *Nat. Bur. Stand.*, 1947, " Tables of selected values of thermodynamic properties ", Series III, Table 8).

q_2 and q_3 refer to the summation processes for which heat data are known (Bichowsky and Rossini, " Thermochemistry of Chemical Substances ", Reinhold, 1936; and direct experiments on mixing methyl iodide and ether in the present calorimeter):

$$\underset{(Et_2O)}{I_2} \xrightarrow{-1{\cdot}6 \pm 0{\cdot}2} \underset{(c)}{I_2} \xrightarrow{18{\cdot}9 \pm 0{\cdot}25} \underset{(gas)}{I_2} + q_2 = 17{\cdot}3 \pm 0{\cdot}4 \text{ kcals.}$$

$$\underset{(gas)}{CH_3I} \xrightarrow{-6{\cdot}5 \pm 0{\cdot}2} \underset{(liq.)}{CH_3I} \xrightarrow{-0{\cdot}1} \underset{(Et_2O)}{CH_3I} + q_3 = -6{\cdot}6 \pm 0{\cdot}2 \text{ kcals.}$$

q_5 arises, as explained, because in the direct calorimetric experiment the gases are saturated with ether vapour. The actual proportion present was determined by absorption with sulphuric acid (monohydrate) in gas-analysis experiments. It was found that on the average at 15° 4·1 c.c. of gaseous product consisted of 1·2 c.c. of ether vapour and 2·9 c.c. of permanent gases, *i.e.*, the volume of ether vapour entrained in the reaction

$$\underset{(Et_2O)}{2CH_3\cdot MgI} + \underset{(Et_2O)}{I_2} \longrightarrow \underset{\substack{(gas + Et_2O \\ \text{vapour at } 15°)}}{C_2H_6} + \underset{(Et_2O)}{2MgI_2} \quad . \quad . \quad . \quad . \quad . \quad (17)$$

is 1·2/2·9 = 0·38 mole per mole of ethane.

The molar heat of vaporisation of ether at 15° is 6·5 kcals. (Int. Crit. Tables).
The probable error is estimated as about 3%, *i.e.*,

$$q_8 = +2{\cdot}7 \pm 0{\cdot}1 \text{ kcals.} \quad \ldots\ldots\ldots (18)$$

Inserting these values, we have

$$\begin{aligned} D(CH_3\text{–}I) = &18{\cdot}2 \pm 0{\cdot}3 \\ &+42{\cdot}2 \pm 1{\cdot}1 \\ &-\ 6{\cdot}3 \pm 1{\cdot}0 \\ &+\ 8{\cdot}6 \pm 0{\cdot}2 \\ &-\ 6{\cdot}6 \pm 0{\cdot}2 \\ &-\ 1{\cdot}35 \pm 0{\cdot}05 \\ \hline = &54{\cdot}75 \pm 1{\cdot}4 \text{ kcals./mole} \quad \ldots\ldots (19) \end{aligned}$$

In the above, the probable errors in the data taken from published values are as shown. The overall probable error in the calorimetric data now obtained is taken as $\sqrt{a^2 + b^2}$ where $a = \pm\sqrt{\Delta^2/n(n-1)}$ for reaction (5) and b is the corresponding value for one half mole of reaction (6).

It can be readily verified that the contribution due to reaction (7) can be neglected, provided the methylmagnesium iodide consumed in reactions (6) and (7) be counted as being all consumed by reaction (6). For this purpose, self-consistent bond data may be taken from Sidgwick ("The Covalent Link in Chemistry", 1933). Suppose two free methyl groups combine as in reaction (6). The heat contribution is

$$2CH_3\cdot \longrightarrow C_2H_6 - 71{\cdot}14 \text{ kcals.} \quad \ldots\ldots\ldots (20)$$

If, on the other hand, the two methyl groups attack the solvent ether, at the hydrogen atom α to oxygen, the successive making or breaking of bonds

$$\left.\begin{aligned} &(a)\ 2CH_3\cdot + 2CH_3\cdot CH_2\text{–}O\text{–}Et \longrightarrow 2CH_4 + 2CH_3\cdot CHO\text{–}Et \\ &(b)\ 2CH_3\cdot CHO\text{–}Et \longrightarrow 2CH_3\cdot CHO + 2C_2H_5\cdot \\ &(c)\ 2C_2H_5\cdot \longrightarrow C_2H_6 + C_2H_4 \end{aligned}\right\} \quad \ldots\ldots (21)$$

involves the following thermochemical contributions if we take the energy of opening one bond in a double bond as half the total for breaking the double bond, in step (*b*) and step (*c*) :

For step (*a*) $-2D(CH_3\text{–}H) + 2D(C_\alpha\text{–}H)$
(*b*) $2D(C\text{–}O) - D(C{=}O)$
(*c*) $D(C\text{–}H) - D(\text{–}CH) - \frac{1}{2}(C{=}C)$

Assuming additive bond strengths, which is a sufficient approximation, the heat change in step (*a*) cancels. Actually the energy required to break the C–H bond in ether is probably rather lower than that to break the C–H bond in methane, but, as will appear, the effect of the approximation is not serious.

The net heat of disposal of the methyl radicals according to reaction (21) is thus

$$\begin{aligned} &2D(C\text{–}O) - D(C{=}O) - \tfrac{1}{2}D(C{=}C) \\ &= 2(76{\cdot}5) - 160 - \tfrac{1}{2}(123) \\ &= -\ 68{\cdot}5 \text{ kcals.} \end{aligned}$$

as compared with −71·14 for the disposal of methyl radicals in forming ethane directly. Although the absolute accuracy of these values may not be high, they are self-consistent, and since reaction (21) occurs to the extent of only 2·5% of reaction (13), the error in neglecting it is of the order $0{\cdot}025 \times (71{\cdot}14 - 68{\cdot}5) = 0{\cdot}07$ kcal., which is inconsiderable compared with the overall probable error.

With the present calorimetric data, a value can also be computed for the bond strength $D(CH_3\text{–}Mg)$. Inserting the values previously used in (10·3), we have $D(CH_3\text{–}Mg) = 60{\cdot}27 + D(Mg\text{–}I) + \frac{1}{2}Q_B$. If we assume $D(Mg\text{–}I)$ is half the heat of formation of magnesium iodide recorded by Bichowsky and Rossini (*op. cit.*, pp. 144, 340), we have $D(Mg\text{–}I) = 68{\cdot}45$ kcals. Such an assumption is not unreasonable if MgI_2 (gas) is mainly covalent in character, as is likely owing to the relative sizes of Mg^{++} and I^-. Then

$$D(CH_3\text{–}Mg) = 60{\cdot}27 + 68{\cdot}46 - 51{\cdot}7 - q_1 - q_4 - \tfrac{1}{2}q_2 - \tfrac{1}{2}q_5.$$

If, further, $q_1 \approx -q_4$, *i.e.*, the heats of solvation of gaseous RMgI and gaseous MgI_2 by ether are taken as equal, and we insert the previously used values for q_2 and q_5, we have

$$D(CH_3\text{–}Mg) = 67{\cdot}0 \text{ kcals.}$$

It is not yet possible to compute the probable error in this result.

Finally, it may be pointed out that the value $D(CH_3\text{–}I) = 54{\cdot}75 \pm 1{\cdot}4$ kcals. is in good agreement with the value of 54·0 kcals. calculated purely from kinetic data by Butler and Polanyi (*Trans. Faraday Soc.*, **1943**, **39**, 19). This must be regarded as substantial support for the kinetic calculations made by these authors.

Further work is in hand to extend the investigation to the reactions $R{\cdot}MgI + HI = RH + MgI_2$ ($R = CH_3$, etc.), since it is unlikely, on theoretical grounds, that the reactions corresponding with (6) and (7) will proceed as smoothly in the case of the higher alkyls, though their suppression by adding excess of magnesium iodide may be effective.

Other halogens are also being investigated in the same way.

QUEEN'S UNIVERSITY, BELFAST. [*Received, July 31st*, 1947.]

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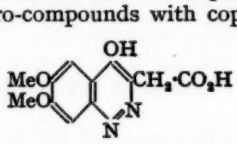
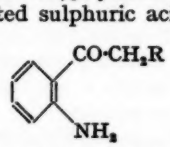
231. *Cinnolines. Part XVIII. The Preparation of 3-Halogeno-4-hydroxycinnolines, and Halogen Exchange Reactions of Diazotised ω-Halogeno-o-aminoacetophenones.*

By K. SCHOFIELD and J. C. E. SIMPSON.

The synthesis of 3-halogeno-4-hydroxycinnolines by cyclisation of diazotised ω-halogeno-*o*-aminoacetophenones, and various concomitant halogen exchange reactions, are described. The bearing of this work and of other new data on the mechanism previously proposed for the formation of 4-hydroxycinnolines from diazotised *o*-aminoacetophenones (Part IV) is discussed, and acid-catalysed enolisation is postulated as an important step in the reaction.

IN Part IV (*J.*, 1945, 520) we described the synthesis of the 4-hydroxycinnoline (I) containing a substituent at C_3, and a number of 4-hydroxy-3-methylcinnolines were subsequently described by Leonard and Boyd (*J. Org. Chem.*, **1946**, **11**, 419) and by Keneford and Simpson (Part XVI, this vol., p. 324). These compounds were all prepared by the same method, *viz.*, diazotisation and cyclisation of appropriate ω-substituted *o*-aminoacetophenones (as II), and it therefore became of interest to see whether similar ring-closures could be effected with compounds of the type (II, R = Cl, Br).

Ruggli and Reichwein (*Helv. Chim. Acta*, **1937**, **20**, 913) have described the preparation of ω-chloro- and ω-bromo-*o*-aminoacetophenones, obtained in 60% yields by reducing the corresponding nitro-compounds with copper and concentrated sulphuric acid. Repetition of

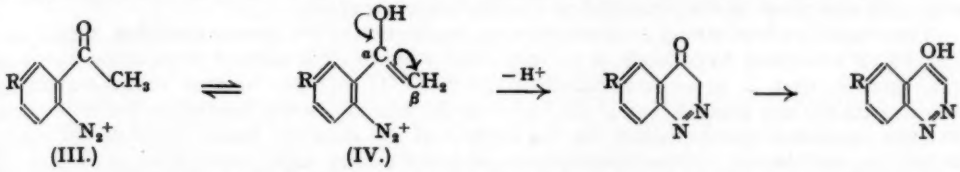
(I.)  (II.) 

this work has shown that the yields claimed are only obtained by rapid isolation of the amine, and has confirmed that the chloro-compound is much the more stable of the two. Two further examples of this reaction have now been examined; bromination of 5-chloro- and 5-bromo-2-nitroacetophenone gave *5-chloro-ω-bromo-* and *5 : ω-dibromo-2-nitroacetophenone*, and subsequent reduction under Ruggli's conditions converted these into the weakly basic *2-amino*-compounds, in 30% and 50% yield respectively. In view of the relationship between the basicity of *o*-aminoacetophenones and cinnoline formation (Part IV, *loc. cit.*), the low basicity and consequent high electrophilic activity of the derived diazonium compounds gave good grounds for expecting efficient ring-closure in these cases. In fact, by diazotising the ω-substituted amines in acetic acid–sulphuric acid, *3-chloro-*, *3-bromo-*, *6-chloro-3-bromo-*, and *3 : 6-dibromo-4-hydroxycinnoline* were obtained in 75—80% yields.

When ω-bromo-*o*-aminoacetophenone was diazotised in presence of hydrochloric acid, the product, again obtained in good yield, differed slightly in melting point from 3-bromo-4-hydroxycinnoline, and a similar difference was noticed between the two *acetyl* derivatives. Analysis revealed that halogen exchange had occurred, the product being *3-chloro-4-hydroxycinnoline*. *3 : 6-Dichloro-* and *3-chloro-6-bromo-4-hydroxycinnoline* were obtained similarly. Cyclisation of diazotised ω-chloro-*o*-aminoacetophenone in presence of hydrobromic acid, followed by acetylation, gave a product which melted between the values for pure 3-bromo-4-acetoxy- and 3-chloro-4-acetoxy-cinnoline, and, although the evidence is not conclusive, it would seem that here too some halogen exchange has taken place. We have not attempted to ascertain the precise stage at which these halogen exchanges occur, but it is likely that they are closely related to

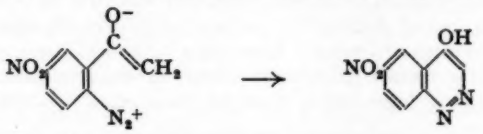
those reported in Part X (*J.*, 1947, 232), and that the diazonium kations are involved. [The halogen exchange observed by Leonard and Boyd (*loc. cit.*) during the chlorination of 6-bromo-4-hydroxycinnoline with a mixture of phosphorus pentachloride and phosphorus oxychloride, a reaction to which there is a parallel in certain 3-bromo-4-hydroxycinnolines (Schofield and Swain, unpublished), is not comparable with the present examples, as it involves a cinnoline nucleus already formed.]

In Part IV (*loc. cit.*) we suggested, on the basis of the data then available, that the synthesis of 4-hydroxycinnolines from *o*-amino-ketones proceeded by the following mechanism :



(III.) (IV.)

Leonard (*Chem. Reviews*, 1945, **37**, 269), on the other hand, suggested that " the reaction probably involves an intramolecular coupling of the diazonium kation with the enolate anion ", and has pointed out that " such a mechanism would be similar to the coupling of an aryldiazonium ion with a phenoxide ion to form an azo compound ". This could hardly be the case, however, under the conditions so far used; coupling of diazonium compounds with phenols, as with



enolic substances in general (*e.g.*, Japp–Klingemann reaction; cf. Linstead and Wang, *J.*, 1937, 807; Leonard, Boyd, and Herbrandson, *J. Org. Chem.*, 1947, **12**, 47), is carried out in alkaline media, and in any case it seems likely that the coupling reagent under such conditions is not the simple diazonium ion (Hodgson and Marsden, *J.*, 1945, 207).

The factors affecting the equilibrium between (III) and (IV), and the relative importance, in any given case, of the electrophilic and nucleophilic activity of the diazonium group and of C_β respectively—insofar as these may be separated at all in such a compact system as the molecule of a diazotised *o*-aminoacetophenone—were not discussed in Part IV because of lack of evidence. Information subsequently accumulated, however, and summarised in the Table,

Cinnoline.	Conditions.	Ref.	Yield, %.
4-Hydroxy-	75% (v/v) H_2SO_4 and AcOH; 10° → 90°	1	≯10
	~3·5N-HCl; room temp.	2	43
	~Conc. HCl; 50—60°	3	70—75
7-Chloro-4-hydroxy-	~5N-HCl; 90°	4	30—35
	~3·5N-H_2SO_4; 28 days at room temp., then 70—80° for 1 hour	2	81
	~Conc. HCl; 50—60°	3	90—95
4-Hydroxy-7-acetyl-	~4N-HCl; 10 → 80°	5	55 of corresponding phenol
	~Conc. HCl; 3 days at room temp., then 75—80° for 2½ hours	5	65
4-Hydroxy-3-methyl-	~2N-HCl; room temp.	2	18
	~Conc. HCl; 50—60°	6	83
4-Hydroxy-7-methyl-	~Conc. HCl; 50—60°	7	60
4-Hydroxy-8-methyl-	~Conc. HCl; 50—60°	8	65
6-Chloro-4-hydroxy-7-methyl-	~5N-HCl; 70—80°	9	90—95
6-Bromo-4-hydroxy-7-methyl-	~2N-HCl; 70—80°	9	35—40 (with 45 of corresponding phenol)

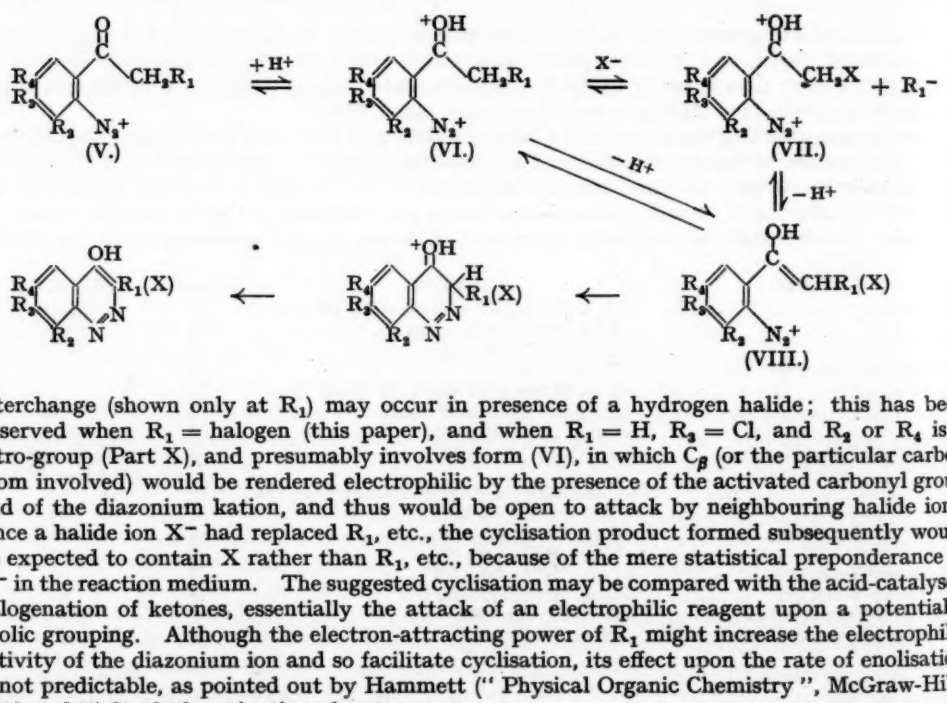
1. Part IV, *loc. cit.*,
2. Leonard and Boyd, *loc. cit.*
3. Keneford and Simpson, Part XIII, *J.*, 1947, 917.
4. Atkinson and Simpson, *J.*, 1947, 232.
5. Schofield and Theobald, unpublished.
6. Keneford and Simpson, Part XVI, *loc. cit.*
7. Keneford and Simpson, unpublished.
8. Morley and Simpson, unpublished.
9. Keneford and Simpson, *J.*, 1947, 227.

throws considerable light on these questions. It is clear from these data that ring-closure of diazotised *o*-aminoacetophenones in which the nucleus is so substituted as to reduce the

electrophilic activity of the diazonium ion occurs only to a small extent in dilute acid solution, but in concentrated acid the reaction is greatly facilitated. The employment of a low temperature also favours the reaction, as is illustrated by the yields for 4-hydroxycinnoline; this has been noted by Leonard and Boyd (*loc. cit.*), but appears to be of secondary importance in comparison with the effect of acid concentration (4-hydroxycinnoline, 4-hydroxy-3-methylcinnoline). The low yield of 6-bromo-4-hydroxy-7-methylcinnoline as compared with that of its 6-chloro-analogue may be partly due to the use of weaker acid in the first case, but it is undoubtedly also caused by the lower electron-attracting power of a bromine atom, as compared with a chlorine atom, in the *p*-position to the diazonium grouping.

These results afford strong evidence that the enolisation of the ketone grouping, suggested in Part IV as a working hypothesis, is an important stage in this method of cinnoline synthesis, and, further, that it is an acid-catalysed process. If suitably located electron-attracting substituents are not present, *i.e.*, if the electrophilic activity of the diazonium ion is relatively low, the reaction depends mainly on the acidity of the medium, which controls the rate of enolisation, and hence, by the development of a sufficiently high nucleophilic activity at C_β, the rate of cyclisation. If, on the other hand, the amino-ketone contains electron-attracting substituents so situated as to exert their maximum effect on the diazonium ion, the activity at that centre is sufficiently enhanced to become the controlling factor, and cyclisation can then be effected in good yield even in dilute acid when the rate of enolisation is low [cf. the high yields of 6-chloro- and 6-bromo-4-hydroxycinnoline obtained in ~2N-acid (Part IV); the yield of 4-hydroxy-6-cyanocinnoline (*loc. cit.*) was not given, but was 70% in both N- and 4N-acid].

On the basis of the accepted mechanism for acid-catalysed enolisation of a carbonyl group (*cf.* Watson, "Modern Theories of Organic Chemistry", 2nd Edition, p. 169) the whole reaction may be represented by the scheme illustrated below, in which cinnoline ring-closure competes with phenol formation arising from one or more of the species (V)—(VIII), and in which group



interchange (shown only at R_1) may occur in presence of a hydrogen halide; this has been observed when R_1 = halogen (this paper), and when R_1 = H, R_3 = Cl, and R_2 or R_4 is a nitro-group (Part X), and presumably involves form (VI), in which C_β (or the particular carbon atom involved) would be rendered electrophilic by the presence of the activated carbonyl group and of the diazonium kation, and thus would be open to attack by neighbouring halide ions. Once a halide ion X^- had replaced R_1, etc., the cyclisation product formed subsequently would be expected to contain X rather than R_1, etc., because of the mere statistical preponderance of X^- in the reaction medium. The suggested cyclisation may be compared with the acid-catalysed halogenation of ketones, essentially the attack of an electrophilic reagent upon a potentially enolic grouping. Although the electron-attracting power of R_1 might increase the electrophilic activity of the diazonium ion and so facilitate cyclisation, its effect upon the rate of enolisation is not predictable, as pointed out by Hammett ("Physical Organic Chemistry", McGraw-Hill, 1940, p. 241) for the bromination of acetone.

We have attempted to investigate one or two cases in which R_1 is a group other than methyl or halogen, but no clear-cut results have emerged. For example, we have previously pointed out (Part IV) that the compound obtained by de Diesbach and Klement (*Helv. Chim. Acta*, 1941, **24**, 158) from the diazotisation of *o*-amino-ω-anilinoacetophenone may have been 3-anilino-4-hydroxycinnoline. We were unable to obtain their compound, and the work of Julian (*J. Amer. Chem. Soc.*, 1945, **67**, 1203) casts some doubt on the general

utility of anilino-compounds in this connection, as apparently isomerisation of the type NHPh·CH(R′)·CO·R ⇌ NHPh·CH(R)·CO·R′ can occur. Having already examined (Part IV) the case of *o* : ω-diaminoacetophenone with negative results, it was natural to investigate the behaviour of *o*-amino-ω-phthalimidoacetophenone (Gabriel and Gerhard, *Ber.*, 1921, **54**, 1067); the product, obtained in *ca.* 40% yield, appears to be the normal phenolic compound. In an effort to modify the course of the reaction, *ω-phthalimido-o-acetamidoacetophenone* was nitrated, but attempts to hydrolyse the *mononitro*-derivative gave a high-melting compound of unknown constitution.

This work is being continued, and derivatives of the new cinnolines will be described later.

Experimental.

(Melting points are uncorrected.)

Diazotisations, unless otherwise stated, were effected at 0° with 5% aqueous sodium nitrite.

ω-Bromo-o-nitroacetophenone.—Pure *o*-nitroacetophenone was prepared by the acetoacetic ester method (Needham and Perkin, *J.*, 1904, **85**, 148; Kermack and Smith, *J.*, 1929, 814) and brominated according to Ruggli and Reichwein (*loc. cit.*); bromination under too powerful illumination (500-watt lamp) gave material which probably contained some of the dibromo-compound, as shown by its unsatisfactory behaviour on reduction.

Diazotisation of ω-Bromo-o-aminoacetophenone.—(*a*) A solution of the amine (2·6 g.) in acetic acid (25 c.c.) and concentrated sulphuric acid (13 c.c.) was diazotised and, after dilution with water (25 c.c.), heated at 70—75° during 30 minutes. The crude product [2 g. (73%), m. p. 275—276°] was recrystallised from ethanol, giving light fawn needles of *3-bromo-4-hydroxycinnoline*, m. p. 276—276·5° (Found : C, 42·5; H, 2·45; N, 12·2; Br, 35·4. $C_8H_5ON_2Br$ requires C, 42·6; H, 2·2; N, 12·45; Br, 35·5%). When 10N-sulphuric acid was used in place of concentrated acid the yield was 56%. Treatment for 2 ours with boiling acetic anhydride, decomposition with water, and crystallisation from ethanol gave *3-bromo-4-acetoxycinnoline*, m. p. 139—140°, as white needles or dense fawn prisms. Satisfactory analytical data could not be obtained for this and certain other compounds (Found : C, 45·8; H, 2·9; N, 10·9. $C_{10}H_7O_2N_2Br$ requires C, 44·9; H, 2·6; N, 10·95%).

(*b*) The suspension obtained from the amine (1 g.), acetic acid (10 c.c.), and concentrated hydrochloric acid (5 c.c.) was diazotised, kept for 1 hour at 70—75°, and the product collected (0·48 g., m. p. 275—276°). Recrystallisation from ethanol gave fawn needles of *3-chloro-4-hydroxycinnoline*, m. p. 278—279° (Found : C, 52·05; H, 2·75; N, 15·9; Cl, 19·7. $C_8H_5ON_2Cl$ requires C, 53·19; H, 2·77; N, 15·52; Cl, 19·7%), identical with the compound described below. *3-Chloro-4-acetoxycinnoline*, prepared as for the bromo-analogue, formed almost colourless needles from ethanol, m. p. 125—126° (Found : C, 53·25; H, 3·2; N, 12·4; Cl, 16·4. $C_{10}H_7O_2N_2Cl$ requires C, 53·9; H, 3·15; N, 12·58; Cl, 15·95%).

Diazotisation of ω-Chloro-o-aminoacetophenone.—(*a*) Diazotisation of the amine (0·83 g.) in acetic acid (5 c.c.) and concentrated hydrochloric acid (5 c.c.) gave a clear solution which, after being left at room temperature until it gave no further coupling with alkaline β-naphthol (2 days), yielded almost pure 3-chloro-4-hydroxycinnoline (84%); cyclisation at 70—75° gave a yield of 66% (Found : N, 15·4; Cl, 20·1%). The acetyl derivative had m. p. 124—125° (Found : N, 13·9; Cl, 16·9%).

(*b*) Replacement of the hydrochloric acid of (*a*) by an equal volume of *ca.* 10N-sulphuric acid in an otherwise similar experiment gave 85% of crude 3-chloro-4-hydroxycinnoline.

(*c*) The amine (0·6 g.) in acetic acid (7·2 c.c.) and *ca.* 10N-sulphuric acid (3·6 c.c.) was diazotised, and the solution poured into *ca.* 10N-hydrobromic acid (5 c.c.) and left at room temperature for 2 days, yielding 56% of a product, m. p. 275—276°; cyclisation at 70—75° (30 minutes) gave 37% of a similar product. Each specimen gave an acetyl derivative, m. p. 130—131°. An approximately 1 : 1 mixture of authentic 3-bromo- (m. p. 276—276·5°) and 3-chloro-4-hydroxycinnoline (m. p. 278—279°) melted at 275—276° after softening at 270°.

5-Chloro-ω-bromo-2-nitroacetophenone.—A solution of 5-chloro-2-nitroacetophenone (10 g.) (Simpson *et al.*, *J.*, 1945, 646) in chloroform (35 c.c.) was treated at 50° with a solution of bromine (8·05 g.) in chloroform (15 c.c.) during 30 minutes, under irradiation from a 200-watt lamp. Removal of solvent under reduced pressure and crystallisation of the crude product (13·8 g., m. p. 93—95°) from ether–ligroin gave soft white needles of *5-chloro-ω-bromo-2-nitroacetophenone*, m. p. 99—99·5° (Found : C, 35·3; H, 2·0; N, 4·8; Cl + Br, 41·7. $C_8H_5O_3NClBr$ requires C, 34·5; H, 1·8; N, 5·0; Cl + Br, 41·5%).

5 : *ω-Dibromo-2-nitroacetophenone.*—5-Bromo-2-nitroacetophenone (10 g.) (Simpson *et al.*, *loc. cit.*) was brominated as described above. Crystallisation of the crude product (13·1 g., m. p. 104—105°) from ether–ligroin gave soft white needles of 5 : *ω-dibromo-2-nitroacetophenone*, m. p. 109—110° (Found : C, 30·3; H, 1·7; Br, 49·1. $C_8H_5O_3NBr_2$ requires C, 29·8; H, 1·6; Br, 49·5%).

5-Chloro-ω-bromo-2-aminoacetophenone.—A solution of the requisite nitro-compound (7 g.) in concentrated sulphuric acid (55 c.c.) was treated at 50° with copper powder (7 g.) during 30 minutes. The mixture was poured on ice, diluted to *ca.* 400 c.c., filtered from copper, and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and concentrated; addition of ligroin gave the unstable amine as yellow needles, m. p. 105—107° (1·95 g.). The substance was not purified further, but was characterised as the *pyridinium* derivative, which (0·27 g.) separated when a solution of the base (0·37 g.) in ethanol (5 c.c.) and pyridine (5 c.c.) was boiled for several minutes; recrystallisation from ethanol gave yellow needles, m. p. 245—246° (decomp.) (Found : N, 9·4; Cl + Br, 34·95. $C_{13}H_{12}ON_2ClBr$ requires N, 8·5; Cl + Br, 35·2%).

5 : *ω-Dibromo-2-aminoacetophenone.*—The appropriate nitro-compound (8 g.) was reduced as above, and gave yellow needles of the amine (3·9 g.), m. p. 110—111°; as before it was not purified further, but was characterised as the *pyridinium* derivative [pale-green plates, m. p. 228—230° (decomp.)] (Found : Br, 40·7. $C_{13}H_{12}ON_2Br_2$ requires Br, 42·9%). Both in this and in the preceding preparation, extraction

of the copper residues with ether gave a second crop of impure amine from which some pure hydroxycinnoline (*q.v.*) could be obtained by diazotisation in sulphuric acid.

Diazotisation of 5-*Chloro-ω-bromo-2-aminoacetophenone.*—(*a*) A solution of the amine (0·93 g.) in acetic acid (9 c.c.) and concentrated sulphuric acid (4·5 c.c.) was diazotised, diluted with water (9 c.c.), and heated at 70—75° for 45 minutes, giving pure 6-*chloro*-3-*bromo*-4-*hydroxycinnoline* (0·79 g.), which separated from ethanol in very light fawn needles, m. p. 311—312° (Found: C, 37·9; H, 2·0; N, 11·1; Cl + Br, 44·8. $C_8H_4ON_2ClBr$ requires C, 37·0; H, 1·6; N, 10·8; Cl + Br, 44·5%). 6-*Chloro*-3-*bromo*-4-*acetoxycinnoline*, from the hydroxy-compound and acetic anhydride (2 hours under reflux), crystallised from ethanol in white prisms, m. p. 166—167° (Found: C, 40·1; H, 1·9; N, 9·05. $C_{10}H_6O_2N_2ClBr$ requires C, 39·8; H, 2·0; N, 9·2%).

(*b*) The solution obtained by diazotising the amine (0·31 g.) in acetic acid (3 c.c.) and concentrated hydrochloric acid (1·5 c.c.) was heated at 70—75° during 30 minutes. The crude 3 : 6-*dichloro*-4-*hydroxycinnoline* which separated (0·24 g., m. p. 298—300°) crystallised from ethanol in light fawn needles, m. p. 305—306° (Found: C, 44·02; H, 2·0; N, 11·9; Cl, 32·5. $C_8H_4ON_2Cl_2$ requires C, 44·70; H, 2·0; N, 13·0; Cl, 32·9%). 3 : 6-*Dichloro*-4-*acetoxycinnoline*, prepared with boiling acetic anhydride, formed white leaflets, m. p. 149—150°, from ethanol (Found: N, 10·5. $C_{10}H_6O_2N_2Cl_2$ requires N, 10·9%).

Diazotisation of 5 : *ω-Dibromo-2-aminoacetophenone.*—(*a*) The amine (1·41 g.) in acetic acid (12 c.c.) and sulphuric acid (7 c.c. of *ca.* 10N) was diazotised, water was added (12 c.c.), and the solution left for 84 hours and then heated at 70—75° for 45 minutes, giving pure 3 : 6-*dibromo*-4-*hydroxycinnoline* (1·1 g.) which crystallised from ethanol in soft, colourless needles, m. p. 315—316° (Found: C, 31·9; H, 2·1; N, 9·1. $C_8H_4ON_2Br_2$ requires C, 31·6; H, 1·3; N, 9·2%). Heating immediately after diazotisation reduced the yield to 0·94 g. 3 : 6-*Dibromo*-4-*acetoxycinnoline* separated from ethanol in colourless prisms, m. p. 176—177° (Found: C, 34·8; H, 2·1. $C_{10}H_6O_2N_2Br_2$ requires C, 34·7; H, 1·8%).

(*b*) Diazotisation of the amine (3·42 g.) in acetic acid (27·5 c.c.) and concentrated hydrochloric acid (17·1 c.c.), followed by heating at 70—75° for 45 minutes, gave pink needles (2·3 g., m. p. 305—307°) of 3-*chloro*-6-*bromo*-4-*hydroxycinnoline*, which had m. p. 310—311° after crystallisation from ethanol (Found: C, 36·4; H, 1·7; N, 10·2. $C_8H_4ON_2ClBr$ requires C, 37·0; H, 1·6; N, 10·8%). Cyclisation at room temperature during 84 hours gave an increased yield of crude product, m. p. 308—309° after recrystallisation, but analysis suggested that halogen-exchange was incomplete (Found: C, 35·6; H, 1·7; N, 11·1. $C_8H_4ON_2ClBr$ requires C, 37·0; H, 1·6; N, 10·8. $C_8H_4ON_2Br_2$ requires C, 31·6; H, 1·3; N, 9·2%). Acetylation of 3-chloro-6-bromo-4-hydroxycinnoline gave 3-*chloro*-6-*bromo*-4-*acetoxycinnoline*; colourless needles from ethanol, m. p. 169—170° (Found: C, 38·8; H, 2·0. $C_{10}H_6O_2N_2ClBr$ requires C, 39·8; H, 2·0%).

Diazotisation of ω-Phthalimido-o-aminoacetophenone.—The suspension obtained from the amine (6 g.) (Gabriel and Gerhard, *loc. cit.*) and concentrated hydrochloric acid (84 c.c.) was treated with water (12 c.c.) and diazotised at 0° with solid sodium nitrite (1·44 g.). Glacial acetic acid (72 c.c.) was added and the suspension kept at 80°. A clear solution was first formed, and after 1 hour the product which gradually separated was collected (2·4 g., m. p. 192—195°) and crystallised from ammoniacal alcohol, giving glistening red-brown prisms and fern-like clusters of, presumably, *ω-phthalimido-o-hydroxyacetophenone*, m. p. 196—198° [Found: C, 66·5; H, 3·6; N, 5·3; *M* (Rast), 288. $C_{16}H_{11}O_4N,\frac{1}{2}H_2O$ requires C, 66·2; H, 4·2; N, 4·8%; *M*, 290].

Nitration of ω-Phthalimido-o-acetamidoacetophenone.—ω-Phthalimido-*o*-aminoacetophenone (0·5 g.) and acetic anhydride (2 c.c.) at 95° rapidly yielded *ω-phthalimido-o-acetamidoacetophenone* (0·53 g.); colourless crystals, m. p. 225—226°, from acetic acid (Found: C, 66·0; H, 4·4; N, 8·8. $C_{18}H_{14}O_4N_2,\frac{1}{2}H_2O$ requires C, 66·15; H, 4·5; N, 8·6%). The acetyl compound (3·3 g.) was added during 20 minutes with stirring to a mixture of nitric acid (15 c.c., *d* 1·48) and concentrated sulphuric acid (6 c.c.) at − 5°. After a further 40 minutes (temperature ⩾ + 5°), the solution was poured on ice, and the solid collected (3·8 g., m. p. 255—260°). Four crystallisations from acetic acid gave almost colourless needles, m. p. 265—270° (decomp.), of x-*nitro-ω-phthalimido-o-acetamidoacetophenone* (Found: C, 58·5; H, 3·6. $C_{18}H_{13}O_6N_3$ requires C, 58·8; H, 3·5%). This compound (0·49 g.) was refluxed with acetic acid (5 c.c.) and concentrated hydrochloric acid (5 c.c.) for 30 minutes; more acetic acid (5 c.c.) was added, and refluxing continued for a further 6 hours, but dissolution did not occur. The *product* (0·37 g.) was collected and crystallised from acetic acid (220 c.c.), from which it formed glistening yellow needles (0·3 g.), darkening at 310° and decomposing at 325—328° (Found: C, 58·8; H, 3·6; N, 13·4. $C_{16}H_{11}O_5N_3$ requires C, 59·1; H, 3·7; N, 12·9%).

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232. *Kationoid Reactivity of Aromatic Compounds. Part VI. The Direct Amination of* meso*Benzanthrone.*

By William Bradley.

The experiments of Part III (*J.*, 1937, 1091) have been extended to include a study of the direct amination of *meso*benzanthrone. Amination occurs when *meso*benzanthrone is heated in dimethylaniline solution with sodamide and oxygen. The principal products of the reaction are 6-*amino*meso*benzanthrone*,* 6-hydroxy*meso*benzanthrone and 6 : 6′-*di*meso*benzanthronylamine*. Indications are obtained of the formation of small amounts of 4-substituted *meso*benzanthrones. The mechanism of these reactions is discussed. The application of the sodamide–oxygen and sodamide–piperidine–oxygen reagents to various types of ketones is described. With the piperidine reagent, anthanthrone yields mainly a *dipiperidino*-derivative.

The direct replacement of hydrogen attached to an aromatic nucleus by the amino-group has been accomplished in several ways. Graebe (*Ber.*, 1901, **34**, 1178) obtained amines in poor yield by treating aromatic hydrocarbons with hydroxylamine hydrochloride in the presence of anhydrous aluminium chloride. De Turski (D.R.-P. 287,756), using a solution of hydroxylamine and ferrous sulphate in concentrated sulphuric acid, converted anthraquinone, benzophenone, and xanthone into related amino-compounds. Using sodamide at 205—235°, Sachs (*Ber.*, 1906, **39**, 3006) obtained 1 : 5-aminonaphthol from α-naphthol, 5-amino-2-naphthol from β-naphthol, 1 : 5-naphthylenediamine from α-naphthylamine, and 2 : 5-naphthylenediamine from β-naphthylamine. A particularly easy replacement of nuclear hydrogen occurs when certain aromatic nitro-compounds are treated with hydroxylamine in alcoholic alkaline solution (cf. *J.*, 1932, 1254). Angeli (*Gazzetta*, 1901, **31**, 27) prepared 4-nitro-1-naphthylamine from α-nitronaphthalene, and later, Meisenheimer and Patzig (*Ber.*, 1906, **39**, 3534) obtained 2-nitro-1-naphthylamine from β-nitronaphthalene, and a mixture of 2 : 4-dinitroaniline and 4 : 6-dinitro-*m*-phenylenediamine from *m*-dinitrobenzene. Further, heating with sodamide transforms many bases of the pyridine–quinoline series into the corresponding amino-derivatives. Pyridine itself affords both α-amino- and αα′-diamino-pyridine (Tschitschibabin and Seide, *J. Russ. Phys. Chem. Soc.*, 1914, **46**, 1216; 1920, **50**, 522), *iso*quinoline affords 1-amino*iso*quinoline (Tschitschibabin and Oparina, *ibid.*, p. 543), and quinoline, α-aminoquinoline (Tschitschibabin and Sazepina, *ibid.*, p. 553).

In other instances aromatic amines have been obtained by adding the elements of ammonia to compounds having the character of quinones. For example, indanthroneazine reacts additively with ammonia to form 4-aminoindanthrone (Scholl and Berblinger, *Ber.*, 1903, **36**, 3438).

Attention has recently been directed to the readiness with which *meso*benzanthrone reacts with such reagents as piperidine, potassium hydroxide, and the Grignard reagent to form substitution products, especially in the presence of an oxidant (Bradley, *J.*, 1937, 1091; Bradley and Jadhav, *ibid.*, p. 1791). In each instance the active substituting agent is the anion of a very weak acid, and the characteristic replacement of hydrogen, situated either *ortho* or *para* to the carbonyl group, is fully in accord with modern theoretical views of the course of aromatic substitution (cf., *e.g.*, Robinson, *J. Soc. Dyers and Col.*, Jubilee Issue, 1934, pp. 65, 73; Bradley and Robinson, *J.*, 1932, 1254). These experiments have now been extended to a study of the direct amination of *meso*benzanthrone. As was expected, the reaction occurred when sodamide was used in the presence of oxygen, the composition of the product being determined by the amount of oxygen available. The reaction was strongly exothermic, and useful results could be obtained only when a diluent such as dimethylaniline was added, which was stable to sodamide over the temperature range employed.

When oxygen is passed rapidly through a stirred suspension of sodamide and *meso*benzanthrone (I) in dimethylaniline a reaction commences at 70—80° with evolution of ammonia and rapid rise in temperature. Sodium nitrite is formed in considerable amount and an organic product which consists almost entirely of 6-*amino*meso*benzanthrone* (V), 6-hydroxy-*meso*benzanthrone (VI), and 6 : 6′-*di*meso*benzanthronylamine* (VIII). No 4-derivative of *meso*benzanthrone could be isolated, but qualitative tests indicated the occurrence of small amounts of 4-amino*meso*benzanthrone (X) and 4-hydroxy*meso*benzanthrone (IX) and the probable presence of 4 : 6′-*di*meso*benzanthronylamine* (XI). There were no indications of the formation either of violanthrone or of polyamino*meso*benzanthrone.

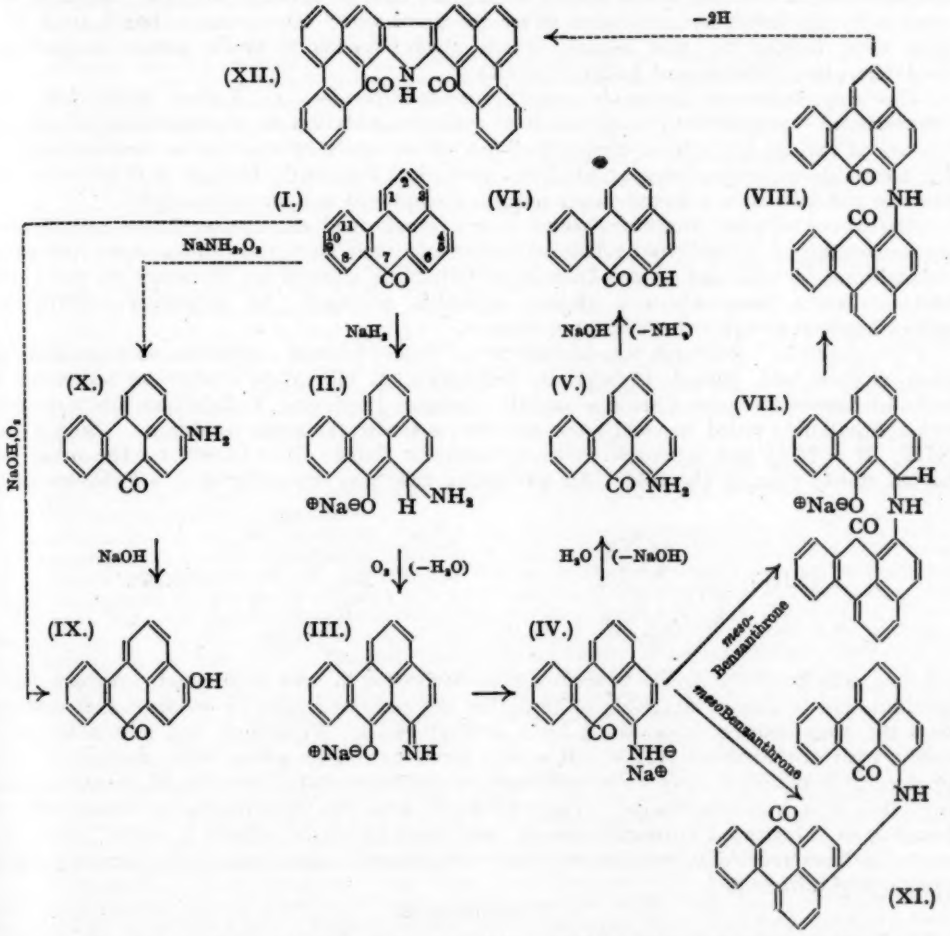
* The numbering of the benzanthrene ring system now used is that shown in (I); that in earlier papers cited was based on anthracene.—Editor.

The constitution of 6-amino*meso*benzanthrone follows from its very easy conversion into 6-hydroxy*meso*benzanthrone (Bradley and Jadhav, *loc. cit.*) when it is heated with amyl-alcoholic potassium hydroxide. Further, it may be synthesised by the action of ammonia on 6-hydroxy-*meso*benzanthrone (Bradley and Jadhav, unpublished experiment). 6-Amino*meso*benzanthrone is very probably identical with the amino*meso*benzanthrone having the same m. p. (185°) which was obtained by Pieroni (*Ann. Chim. appl.*, 1931, **21**, 155) by the action of sodamide on *meso*benzanthrone in toluene. The constitution of the product regarded as 6 : 6′-di*meso*-benzanthronylamine was indicated by the formation of both 6-amino- and 6-hydroxy-*meso*-benzanthrone when it was hydrolysed by means of alcoholic potassium hydroxide. It was synthesised by condensing 6-amino- with 6-chloro-*meso*benzanthrone. The purification of the di*meso*benzanthronylamine prepared directly from *meso*benzanthrone and sodamide was rendered difficult because of the presence of another product having a similar range of solubilities. The composition of the partly purified product was that of a homogeneous di*meso*benzanthronylamine, which suggests that the second product is either an isomeride of the 6 : 6′-derivative of the *carbazole* (XII) formed from 6 : 6′-di*meso*benzanthronylamine by dehydrogenation. Carbazole formation is commonly observed when anthrimides are heated with alkalis (cf. D.R.-PP. 262,788, 267,522; Friedlander, " Fortschritte in der Teerfarbenfabrikation ", Vol. II, pp. 618, 620), and it is possible that a portion of the crude product is of this type. An isomeride of 6 : 6′-di*meso*benzanthronylamine is present since the crude di*meso*benzanthronylamine is hydrolysed by heating with alcoholic potassium hydroxide to a mixture of amines and phenols. The amines show the characteristic property of 4-amino*meso*benzanthrone of dissolving in alcohol to form a yellow solution with a green fluorescence; 6-amino*meso*benzanthrone gives a yellow non-fluorescent solution. The phenols dissolve in dilute sodium hydroxide with a brownish-yellow colour and an intense green fluorescence which is characteristic of 4-hydroxy*meso*benzanthrone; 6-hydroxy*meso*benzanthrone forms a yellow, non-fluorescent solution. These are the scission products to be expected of either 4 : 6′- or 4 : 4′-di*meso*benz-anthronylamine. Actually the former isomeride is the more likely possibility because the di*meso*benzanthronylamines are formed by the interaction of *meso*benzanthrone with the anion of 6-amino*meso*benzanthrone (see below), a process which can yield only a mixture of the 6 : 6′- and 4 : 6′-forms.

The amines encountered in the amination of *meso*benzanthrone show interesting differences in behaviour when a drop of methyl-alcoholic potassium hydroxide is added to their solutions in dry pyridine (Bradley, *J. Soc. Dyers Col.*, 1942, **58**, 2). 4-Amino*meso*benzanthrone, like β-aminoanthraquinone, readily forms an alkali derivative whose solution in pyridine is orange. 6-Amino*meso*benzanthrone resembles α-aminoanthraquinone in that the colour of its pyridine solution remains unaltered on adding the alkali. The acetyl derivatives of both the amino*meso*benzanthrones form potassium salts quite readily. Both 6 : 6′- and 4 : 6′-di*meso*-benzanthronylamine form deep bluish-green potassium salts which resemble the analogous derivatives of 1 : 1′- and 1 : 2′-dianthraquinonylamine (Bradley, *loc. cit.*).

Steps in the Substitution Process.—When oxygen is present in sufficient excess the first step in the reaction between sodamide and *meso*benzanthrone probably involves the attack of amide ions on free *meso*benzanthrone at a carbon atom situated in the *ortho*-position relative to the carbonyl group, an orientation which corresponds with that of the basic group in the products formed by adding ammonia or primary or secondary amines to αβ-unsaturated ketones. A complex ion (II) is formed which then by further change yields 6-amino*meso*benzanthrone (V). There appear to be two possible courses of the second stage in the formation of this compound; (*a*) the complex anion is oxidised directly to 6-amino*meso*benzanthrone and a hydroxyl ion, or (*b*) the complex anion is first dehydrogenated by oxygen to the anion of 6-amino*meso*benz-anthrone (III, IV) which subsequently reacts with water to form a hydroxyl ion and the free base. A decision in favour of the second hypothesis is indicated by the following considerations. In the first place, sodamide and oxygen react by an independent process to form sodium nitrite (cf. Bergstrom and Fernelius, *Chem. Reviews*, 1933, **12**, 43) which, in fact, is always encountered amongst the products of the reaction. Increase in the rate of passing oxygen should decrease the proportion of sodamide which is available for the substitution process. Secondly, increase in the oxygen rate reduces the yields of both 6-amino- and 6-hydroxy-*meso*benzanthrone (VI) but notably increases the yield of 6 : 6′-di*meso*benzanthronylamine. Since the last must be generated by the interaction of the anion of 6-amino*meso*benzanthrone with *meso*benzanthrone by a process analogous to that which yields the complex ion (II), it follows that the anion of 6-amino*meso*benzanthrone is not formed in the substitution process by the interaction of 6-amino*meso*benzanthrone with sodamide but results directly from the complex anion (II) by

dehydrogenation. A notable feature of the direct amination of *meso*benzanthrone is the marked preponderance of substitution *ortho* to the carbonyl group. Piperidination on the contrary yields almost exclusively, if not entirely, the *para*-derivative (Bradley, *J.*, 1937, 1091). The circumstances which control the type of substitution are doubtless complex, and it is easy to demonstrate that several possible contributory factors are in themselves insufficient. One of



these is the composition of the attacking anion; whilst *meso*benzanthrone is hydroxylated principally *para* to the carbonyl group, nitrobenzene yields mainly *ortho*-nitrophenol (Bradley and Jadhav, *loc. cit.*). Another is the co-ordinating power of the anion; the order is alkyl > NC_5H_{10} > NH_2 > OH, yet sodamide and the Grignard reagent (Charrier and Ghigi, *Gazzetta*, 1932, **62**, 928; Allen and Overbaugh, *J. Amer. Chem. Soc.*, 1935, **57**, 740) yield *ortho*-derivatives while sodiopiperidine and potassium hydroxide form *para*. Steric factors are frequently involved, but in the present instance the bulky anion of 6-amino*meso*benzanthrone reacts in the same sense as the amide ion and unlike the piperidine ion whose size is intermediate. The decisive factor which regulates the type of substitution is most probably the character of the complex anion formed at the first stage of the substitution process. Energy considerations will favour the formation of one particular form of the anion. Further, one form will be more easily dehydrogenated to a stable aromatic configuration than any other. The first of these factors is the more significant. A general consideration of the results of hydrogen replacement by anions in typical aromatic compounds activated by C=O, C=N or NO_2 groups shows that hydroaromatic products are only rarely encountered. The rate-determining step which fixes the type of substitution whether *ortho* or *para* is thus the first, since the dehydrogenation of the complex anion initially formed is always an easier process. This view is also in agreement with

general theoretical requirements, the effect of the initial anion-attack being to modify the stable aromatic nucleus to a hydroaromatic form which is unlikely to possess the same high resonance energy, while dehydrogenation of the complex anion reconstitutes the aromatic form.

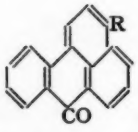
A point of interest is the high yield of 6-hydroxy*meso*benzanthrone obtained by the action of sodamide on *meso*benzanthrone. This phenol arises by the hydrolysis of 6-amino*meso*-benzanthrone, a reaction which occurs with great ease in alkaline solution. An alternative route is by the direct hydroxylation of *meso*benzanthrone; but no more than a trace should have been formed by this means, which always yields a much higher proportion of the 4-derivative (Bradley and Jadhav, *loc. cit.*).

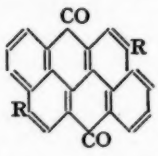
The Application of Sodamide and Sodamide–Piperidine to Ketones other than meso-*Benzanthrone.*—Attempts to bring about the direct amidation or piperidination of aromatic ketones of various types have shown that few are so easily substituted as *meso*benzanthrone. The sodamide–piperidine reagent has been used more frequently because it is generally more effective and safer than sodamide when oxygen is employed as hydrogen-acceptor.

Amination. Under conditions which easily transformed *meso*benzanthrone into 6-amino-*meso*benzanthrone, pyranthrone remained completely unchanged, nor could a basic derivative of violanthrone be obtained. *trans*-Dibenzpyrenequinone showed no tendency to react below 140°, at which temperature a violent explosion occurred. An explosion occurred with anthanthrone at a slightly lower temperature.

Piperidination. Although *meso*benzanthrone readily formed 4-piperidino*meso*benzanthrone when oxygen was passed through its suspension in piperidine containing sodamide, yet 1 : 3 : 5-tribenzoylbenzene, phenyl α-naphthyl ketone, fluorenone, 1 : 2-benzanthraquinone and *peri*naphthalimide failed to yield basic derivatives under the same conditions. Both 3-nitro- (XIII; R = NO_2) and 3-benzoyl-*meso*benzanthrone (XIII; R = COPh) on the other hand reacted slowly even in the cold. An interesting case was encountered in 4-methyl*meso*benz-

(XIII.)





(XIV.)

anthrone, which reacted slowly to form a basic derivative in very small yield. Apparently the piperidide ion is almost incapable of replacing the *ortho*-hydrogen of *meso*benzanthrone even when the *para*-position is occupied by a methyl group. Piperidine and sodamide reacted readily with anthanthrone (XIV; R = H), forming a deep violet basic derivative which appeared to be mainly a *dipiperidino-anthanthrone* [probably (XIV, R = NC_5H_{10})] with a smaller proportion of a mono-derivative. These products were not investigated in detail but their character as substituted anthanthrones is clear since they are soluble in acids, insoluble in aqueous sodium hydroxide, but soluble with a violet colour in the same alkali containing sodium hyposulphite (dithionite).

Experimental.

The Reaction between Sodamide and meso*Benzanthrone in the Presence of Oxygen.*—A rapid current of oxygen was passed through a stirred suspension of sodamide (16 g.) and *meso*benzanthrone (50 g.) in dry dimethylaniline (200 c.c.), access of moisture being prevented. The mixture was heated in an oil-bath. An exothermic reaction commenced at 70° with evolution of ammonia, and the colour of the suspension changed from brown, through green to black. The inner temperature was kept at 130—140° for an hour, and then the product was cooled and stirred with water.

Larger amounts of *meso*benzanthrone could be treated if, when the first exothermic reaction was almost complete, the temperature was reduced to 90°, and further quantities of sodamide (16 g.), *meso*benzanthrone (50 g.), and dimethylaniline (100 c.c.) were added, the stirring being continued until the second exothermic change was complete. Further additions could be made in the same manner. At least 200 g. of *meso*benzanthrone could be treated without loss of yield or purity. When a large amount of *meso*benzanthrone was treated in a single operation, a violent, uncontrollable reaction ensued.

The water and dimethylaniline solutions were filtered from undissolved material, and this was extracted alternately with benzene and water. The residue (10·5 g.) was a friable, reddish-brown powder, (A).

The benzene and dimethylaniline solutions were combined, and were then washed with water. The washings were added to the aqueous solutions already obtained. The united aqueous solutions were brown and showed a green fluorescence. Nitrites were present in considerable amount; when the solution was acidified, brown nitrous fumes were evolved. Acidification in the presence of urea (50 g.) gave an ochre-yellow solid (2·5 g., m. p. 138—142°), from which 1·5 g. of crude 6-hydroxy*meso*benz-anthrone were extracted by treatment with benzene. The residue was a dark brown powder, m. p. 180—220°. It could not be obtained in a homogeneous condition by either crystallisation or sublimation.

4-Hydroxy*meso*benzanthrone was present (fluorescence); a portion of the material was soluble in cold sodium hydrogen carbonate solution, a circumstance suggesting the presence of carboxylic acids.

The combined benzene and dimethylaniline solutions were evaporated on the water-bath, and concentrated hydrochloric acid was added to the residual oil. A deep orange solution was obtained. The undissolved material was further treated with hot concentrated hydrochloric acid until nothing more dissolved. The united acid solutions, filtered through glass-wool, cooled, and then added to water, gave an abundant canary-yellow precipitate (19·2 g., m. p. 165—168°). Recrystallisation from alcohol gave golden-yellow prisms, m. p. 186°, consisting of 6-*amino*mesobenzanthrone (Found: C, 83·5; H, 4·3; N, 5·5. $C_{17}H_{11}ON$ requires C, 83·2; H, 4·5; N, 5·7%). Whilst the pure base dissolved in alcohol to a yellow non-fluorescent solution, the crude base exhibited a green fluorescence, a circumstance indicating the probable presence of 4-amino*meso*benzanthrone. The amount of the latter, if present, was no doubt quite small, since none could be isolated by fractional crystallisation or adsorption of the crude base. A further indication of its presence was given when 1 g. of the oily material, which resulted when the final mother-liquors were evaporated completely, was fused at 200—240° for 30 minutes with 10 g. of potassium hydroxide and 1 c.c. of water. The product was partly soluble in water with a brown colour and a green fluorescence (hydrolysis to 4-hydroxy*meso*benzanthrone?). Determinations of the nitrogen content of the crude base (Found: N, 5·3, 5·3, 5·5%) excluded the presence of polyamino*meso*benzanthrones, and fractional extraction of the crude base by means of 10% hydrochloric acid, in which 6-amino*meso*benzanthrone dissolved hot to a lemon-yellow solution but separated almost completely on cooling, disclosed no constituent more strongly basic than 6-amino*meso*benzanthrone itself.

6-Aminomesobenzanthrone dissolves in concentrated sulphuric acid with a bright cherry-red colour, which is easily distinguished from the orange of 4-amino*meso*benzanthrone in the same solvent.

Hydrolysis of 6-*amino*mesobenzanthrone *to* 6-*hydroxy*mesobenzanthrone. 1 G. of the base was boiled with a suspension of potassium hydroxide (7 g.) in amyl alcohol (30 c.c.). A copious crystalline yellow precipitate separated quickly. After 1 hour, this was collected, washed with alcohol and then with water, and finally extracted by means of hot concentrated hydrochloric acid, giving a residue (0·4 g.), whose m. p. (176°) was not depressed by pure 6-hydroxy*meso*benzanthrone (m. p. 178°), obtained by heating 1-benzoyl-2-naphthol with aluminium chloride (Bradley and Jadhav, *loc. cit.*). The evaporated amyl-alcoholic mother-liquor gave 0·35 g. of similar material, m. p. 171°.

6-*Acetamido*mesobenzanthrone. This *derivative* separated when the base (1 g.) was boiled for an hour with 20 c.c. of acetic anhydride. It crystallised from glacial acetic acid in slender yellow prisms, m. p. 223° (Found: N, 4·9. $C_{20}H_{13}O_2N$ requires N, 4·9%). It gave an orange solution in concentrated sulphuric acid with a yellow fluorescence.

The Neutral Fraction of the Amination Product.—(a) 6-*Hydroxy*mesobenzanthrone. The brownish-yellow substance (23 g.), which remained when the evaporated benzene–dimethylaniline solution had been treated with hydrochloric acid, contained neither 4 : 4′-di*meso*benzanthronyl nor violanthrone. When it was treated with benzene, all but a very small amount dissolved readily, and the concentrated solution gave 20·5 g. of crude 6-hydroxy*meso*benzanthrone (m. p. 150—152°), together with 2 g. of dark substance, m. p. 80—90°. Recrystallisation from glacial acetic acid gave pure 6-hydroxy*meso*benzanthrone, m. p. 176—178°. The mother-liquors contained *meso*benzanthrone, indicated by formation of violanthrone when 0·5 g. of the recovered solute was fused for 15 minutes at 240° with 10 g. of potassium hydroxide and 1 c.c. of water. It was possible to estimate the amount of 6-hydroxy*meso*benzanthrone in the neutral fraction by taking advantage of its ready formation of a boroacetate (cf. *J.*, 1937, 1791), and of the sparing solubility of this derivative in acetic anhydride. When 10 g. of pure 6-hydroxy*meso*benzanthrone dissolved in 60 c.c. of acetic anhydride were boiled for an hour with a solution of boroacetic anhydride, prepared from boric acid (20 g.) and acetic anhydride (100 c.c.), 14·6 g. of the boroacetate, m. p. 262—264° (decomp.) (*i.e.*, 96% of the theoretical yield), separated from the partly evaporated solution on cooling and standing. Treated similarly, 10 g. of the benzene-soluble fraction of the neutral product gave 9·2 g. of the boroacetate (m. p. 262—264°, decomp.). The amount of 6-hydroxy*meso*benzanthrone present in the whole of the reaction product was therefore 13—14 g.

(b) 6 : 6′-*Di*mesobenzanthronylamine. The reddish-brown product (A) was extracted successively with 10% sodium hydroxide solution and with hot concentrated hydrochloric acid. The undissolved portion (10 g., m. p. 310—340°. Found: N, 2·9. Calc. for $C_{34}H_{17}O_2N$: N, 3·0%) was treated with boiling glacial acetic acid, which dissolved only traces of the substance, and then crystallised from nitrobenzene. A product, m. p. 360—365°, was readily obtained, but further purification was difficult. Ultimately, brownish-red prisms, m. p. 380—382° (Found: N, 3·4, 3·2. $C_{34}H_{17}O_2N$ requires N, 3·0%), separated, which were identical with the 6 : 6′-*di*mesobenzanthronylamine prepared by heating together 6-amino- and 6-chloro-*meso*benzanthrone.

A solution of 6-chloro*meso*benzanthrone (9 g.) and 6-amino*meso*benzanthrone (8·5 g.) in nitrobenzene (30 c.c.) was boiled for 4 hours. Even in the absence of any condensing agent the initial yellow colour of the solution changed rapidly through orange to deep red. Thin, reddish-bronze prisms separated on cooling, and these, recrystallised from nitrobenzene, gave a pure product, m. p. 382° (Found: N, 3·3. $C_{34}H_{17}O_2N$ requires N, 3·0%). 6 : 6′-Di*meso*benzanthronylamine gives a reddish streak with a violet tinge when rubbed on porcelain. Solutions in concentrated sulphuric acid are blue in thin and reddish-violet in thick layers; they show a faint reddish fluorescence. It is slightly soluble in hot acetic anhydride to a brownish-yellow solution with a pink tinge, which becomes deep reddish-orange, violet in thin layers, when boroacetic anhydride is added. It dissolves sparingly in hot propyl alcohol, giving a rose-yellow solution which becomes bluish-grey with propyl-alcoholic potassium hydroxide, and pale green on the further addition of pyridine. It is rather more soluble in hot amyl alcohol. The yellow solution becomes deep emerald-green with hot amyl-alcoholic potassium hydroxide, but the original colour is restored on cooling or even on dilution with hot amyl alcohol. The brownish-yellow solution in dry pyridine shows no immediate change when solid potassium hydroxide is added, but on warming, a deep brownish-green colour develops and a green precipitate forms on standing. A deep emerald-green colour is also obtained when methyl-alcoholic potassium hydroxide is added to a solution in pyridine; sodium causes a similar change of colour and ultimately a green precipitate.

4-*Iodo*meso*benzanthrone*. 4-Amino*meso*benzanthrone was diazotised by dissolving 12·5 g. in 50 c.c. of cold, concentrated sulphuric acid and adding the solution as a thin stream to a stirred solution of sodium nitrite (4 g.) in concentrated sulphuric acid (25 c.c.), the temperature being kept below 10°. After 30 mins.' standing, the solution was added to ice-water and the slight excess of nitrous acid decomposed by adding urea. The precipitated diazonium salt was collected, suspended in water (250 c.c.) and stirred with a solution of potassium iodide (25 g.) in water (75 c.c.). Crude 4-*iodo*meso*benzanthrone* was precipitated as a granular powder, m. p. 158—160° with slight previous softening. Crystallisation from alcohol raised the m. p. to 164—165° (Found: C, 57·6; H, 2·6. $C_{17}H_9OI$ requires C, 57·3; H, 2·6%).

4-*Methylamino*meso*benzanthrone*.—A suspension of 4-iodo*meso*benzanthrone (0·9 g.) in 15 c.c. of 33% (w/v) aqueous methylamine was heated at 200° during 6 hours. The product consisted of a pale yellow solution containing a small quantity of 4-hydroxy*meso*benzanthrone and dark, brownish-red crystals which exhibited a marked green lustre. These were extracted with alcohol, giving a yellow solution and a small granular residue, m. p. 270—320°. The solution yielded slender, golden-yellow prisms, m. p. 223—224° (Found: N, 5·2. $C_{18}H_{13}ON$ requires N, 5·4%), of 4-*methylamino*meso*benzanthrone*, showing a bluish-green fluorescence in alcohol.

4 : 6′-*Di*meso*benzanthronylamine*.—4-Iodo*meso*benzanthrone (2·6 g.) and 6-amino*meso*benzanthrone (1·8 g.) were heated together in boiling nitrobenzene (15 c.c.) with the addition of a trace of copper powder. (There was no reaction in the absence of copper.) The colour of the solution quickly changed from yellow to red, and after 10 hours it was filtered, cooled, and then diluted with alcohol. The crystalline precipitate was collected, and exhaustively extracted with hot alcohol. The residue was crystallised first from glacial acetic acid and then from pyridine, giving orange-red prisms with a bronze lustre, m. p. 395° (Found: N, 3·0. $C_{34}H_{17}O_2N$ requires N, 3·0%). 4 : 6′-*Di*meso*benzanthronylamine* gives an orange streak with a greenish-yellow lustre when rubbed on porcelain. Its solution in concentrated sulphuric acid is reddish-violet in thick, and blue in thin layers. It dissolves in acetic anhydride with a golden-yellow colour which remains quite unaltered when boroacetic anhydride is added. A solution in hot amyl alcohol is yellow without pink tinge, and a weak olive-green colour develops when amyl-alcoholic potassium hydroxide is added. It is very sparingly soluble in hot propyl alcohol to a yellow solution, which shows no change of colour when hot propyl-alcoholic potassium hydroxide is added, or any on the further addition of pyridine. A yellow solution in dry pyridine, however, gives an immediate deep blue-green coloration with a drop of methyl-alcoholic potassium hydroxide.

Action of Potassium Hydroxide on Various Products.—(1) 6 : 6′-*Di*meso*benzanthronylamine*. (*a*) 1 G. of the amine was boiled for 2 hours with a solution of potassium hydroxide (5 g.) in 20 c.c. of amyl alcohol. The maroon suspension rapidly became bluish-green and finally yellowish-green with evolution of ammonia. Water was then added, and the amyl alcohol removed in steam. The suspension so obtained was kept for 12 hours, and then filtered. The acidified filtrate afforded a flocculent yellow precipitate (0·1 g., m. p. 165—168°) which gave pure 6-hydroxy*meso*benzanthrone when crystallised from benzene. The alkali-insoluble portion was taken up in benzene, and recovered from the dried solution by evaporating the solvent. The black tarry residue, taken up in 10 c.c. of acetic anhydride and then boiled for an hour with boroacetic anhydride (from 3 g. of boric acid and 15 c.c. of acetic anhydride), gave 0·3 g. of the boroacetate of 6-hydroxy*meso*benzanthrone, m. p. 225—227°.

(*b*) A solution of potassium hydroxide (10 g.) in ethyl alcohol (40 c.c.) was added to a suspension of 6 : 6′-di*meso*benzanthronylamine (1·6 g.) in pyridine (15 c.c.), and the mixture boiled for 30 hours. A green solution formed slowly, and this finally became almost yellow. It was then added to water and rendered acid to Congo-red. A yellow precipitate formed (1·35 g., m. p. 138—148°). It was extracted by means of hot concentrated hydrochloric acid, affording a yellow solution from which 0·4 g. of 6-amino*meso*benzanthrone, m. p. 165—168°, separated when water was added. The acid-insoluble portion of the product (1·1 g., m. p. 156—160°) was nearly pure 6-hydroxy*meso*benzanthrone.

(*c*) Finely powdered 6 : 6′-di*meso*benzanthronylamine (1 g.) was stirred into a melt of potassium hydroxide (50 g.) and water (5 g.) at 200°, and then the temperature was slowly raised to 280° during 1 hour. The product was a deep violet paste. It dissolved partly in water to a violet solution from which a brownish-violet solid separated on exposure to air. The supernatant liquid was yellowish-brown and non-fluorescent; only a trace of material separated when it was acidified. The brownish-violet product, although quite insoluble in water and alkalis, dissolved readily in alkaline sodium hyposulphite (dithionite) solution with a permangate-red colour. In pyridine, xylene, or glacial acetic acid it dissolved sparingly with a brownish-yellow colour, and in concentrated sulphuric acid to a violet solution; in no instance was a fluorescence observed.

(2) 4 : 6′-*Di*meso*benzanthronylamine*. 0·1 G. of 4 : 6′-di*meso*benzanthronylamine was added to a boiling solution of potassium hydroxide (5 g.) in propyl alcohol (20 c.c.). Within a few minutes a portion of the amine had dissolved to a bluish-green solution which changed to a yellow with a green fluorescence. The products of the reaction could not be isolated in a state of purity. When water was added and the propyl alcohol was distilled off, the resulting aqueous solution was brownish-yellow with an intense green fluorescence (4-hydroxy*meso*benzanthrone ?).

(3) *The neutral amination product, m. p.* 310—340°. A suspension of 3 g. of this product in 30 c.c. of pyridine was boiled for 16 hours with a solution of potassium hydroxide (40 g.) in ethyl alcohol (80 c.c.). A bluish-green solution formed and this changed to brownish-yellow. Ammonia was evolved. Water was added, giving a brownish-yellow alkaline solution with an intense green fluorescence, and an insoluble product. A yellow precipitate formed when the aqueous solution was acidified (4-hydroxy*meso*benzanthrone ?), but the quantity obtained was too small to allow of purification. The insoluble product was partly soluble in hot concentrated hydrochloric acid to a yellow solution from which a brownish-yellow basic substance (0·3 g., m. p. 155—160°) separated when water was added. It appeared to be essentially a mixture of 4- and 6-amino*meso*benzanthrones (Found: N, 4·9, 5·0. Calc. for $C_{17}H_{11}ON$: N, 5·7%). It dissolved in alcohol to a yellow solution with an intense green fluorescence (4-amino*meso*benzanthrone), and in acetic anhydride with a yellow colour becoming brown and developing an intense green fluorescence when boroacetic anhydride was added (6-amino*meso*benzanthrone). When it was

boiled for an hour with acetic anhydride (10 c.c.), and the solution cooled, a bronzy mass, m. p. 208—215°, separated. Recrystallisation from glacial acetic acid gave pure 6-acetamido*meso*benzanthrone.

The neutral fraction of the reaction product was partly soluble in benzene; an oily solid remained when the solution was evaporated. Treatment with glacial acetic acid gave pure 6-hydroxy*meso*-benzanthrone. The benzene-insoluble product was insoluble also in hot glacial acetic acid. It was a dark brown powder, m. p. above 400° (Found: N, 2·4%), which dissolved in concentrated sulphuric acid with a brown colour, and in acetic anhydride to a brownish-yellow solution unchanged on addition of boroacetic anhydride.

Absorption Spectra of the Dimesobenzanthronylamines.—Both the 4 : 6′- and the 6 : 6′-derivative are very sparingly soluble in cold solvents. For the measurements, a 0·01% solution of the former and 0·01% and 0·05% solutions of the latter in pyridine were used. The 4 : 6′-derivative exhibited a broad absorption band extending from the violet to about 5600—5700 A., beyond which the absorption was very small and without characteristic features. The 6 : 6′-isomer showed a similar band which passed through a minimum at about 5800 A. There was a rounded maximum at 6175 A., beyond which the absorption decreased.

Action of Sodamide and Piperidine on Anthanthrone.—A suspension of finely powdered anthanthrone (5 g.) and sodamide (2·5 g.) in piperidine (40 c.c.) was agitated by means of a current of oxygen while being heated under reflux in an oil-bath. The mixture which was initially orange changed to brownish-red. The inner temperature was raised from 100° to 130° during 2 hours and then the semi-solid product was cooled and mixed with ice and water. Concentrated hydrochloric acid was added, and the red solution was separated from the undissolved material, which was again extracted with hot 20% hydrochloric acid. Ultimately 3·4 g. of residue remained; this was mainly unchanged anthanthrone. Addition of ammonia to the filtered acid extracts gave 1·7 g. of deep violet basic product. This was taken up in hydrochloric acid and fractionally reprecipitated by adding sodium acetate. No significant difference in composition could be detected between the first and the final fraction [Found (respectively): N, 5·2, 5·4. $C_{38}H_{38}O_2N_2$ (*dipiperidinoanthanthrone*) requires N, 5·9%. $C_{27}H_{19}O_2N$ (*monopiperidinoanthanthrone*) requires N, 3·6%). The two fractions behaved identically towards solvents and chemical reagents. They were sparingly soluble in alcohol but more easily in pyridine, forming violet solutions. They were insoluble in dilute sodium hydroxide but dissolved with a violet colour when sodium hyposulphite (dithionite) was added. The sodium salt of the reduction product was in each case sparingly soluble in aqueous sodium hydroxide of greater than 1% concentration.

The experiments reported above were carried out in the Department of Applied Chemistry, College of Technology, Manchester. The author is indebted to Professor J. Kenner, F.R.S., who afforded facilities for the work, and to Messrs. Imperial Chemical Industries Ltd. (Dyestuffs Group) for grants.

HIGHGATE, LONDON. [*Received, August 18th*, 1947.]

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233. *The Conversion of Mepacrine and Similar Derivatives of 5-Aminoacridines into Thioacridones by the Action of Hydrogen Sulphide.*

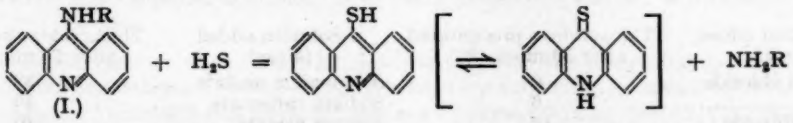
By R. S. ASQUITH, D. LL. HAMMICK, and P. L. WILLIAMS.

Mepacrine and similar derivatives of 5-aminoacridine of the type (I) react with hydrogen sulphide in alkaline, alcoholic solution to give the corresponding thioacridones when the group R is capable of exerting a + *I* effect.

IN the course of an attempt to prepare a dithiocarbamate by the action of carbon disulphide and ammonia on an alcoholic solution of mepacrine, the slow deposition of a red crystalline compound, m. p. 254° (uncorr.), was observed. This was shown to be 2-chloro-7-methoxythioacridone by analysis and by comparison (mixed m. p.) with a specimen prepared by the method used by Edinger and Ritsema (*J. pr. Chem.*, 1903, **68**, 88) for the preparation of thioacridone. 2-Chloro-7-methoxythioacridone has also been obtained by Das Gupta (*J. Indian Chem. Soc.*, 1940, 244) by the action of potassium xanthate on 2 : 5-dichloro-7-methoxyacridine in phenol at 115°; he gives m. p. 245° (uncorr.).

Further investigation has shown that the thioacridone can readily be prepared by passing hydrogen sulphide into alcoholic solutions of mepacrine containing ammonia, sodium hydroxide, or other basic substances. We have also studied the reaction with hydrogen sulphide under these conditions with other derivatives of 2-chloro-5-amino-7-methoxyacridine and of 5-aminoacridine of the general formula (I) as well as with the corresponding 5-chloroacridines.

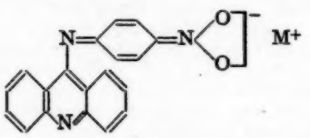
We have established the fact that the reaction is essentially:



by isolating the amine as well as the thioacridone in the case where R is *p*-C_6H_4·OMe.

We also find that, although the reaction is little influenced by the substitution of chlorine and methoxyl groups in the 2 and the 7 position in the acridine nucleus, it depends markedly on the nature of the substituent R in the 5-amino-group. Thus when R is $CHMe{\cdot}[CH_2]_3{\cdot}NEt_2$, $[CH_2]_3{\cdot}OH$, Me, or p-$C_6H_4{\cdot}OMe$, the thioacridone is produced under comparable conditions in yields of 80—90% within 3 hours at room temperature. When R is p-$C_6H_4{\cdot}NO_2$, the reaction is slower, 20% yields being obtained after 24 hours. When, however, R is H (as in 5-aminoacridine and 2-chloro-5-amino-7-methoxyacridine), COMe, Ph, or m-$C_6H_4{\cdot}NO_2$, conversion into thioacridone cannot be brought about (*e.g.*, by hydrogen sulphide in alcoholic alkalis, heating with sodium sulphide in alcohol, fusion with sodium sulphide at 180°). Both 5-chloroacridines react readily with hydrogen sulphide in alkaline alcohol.

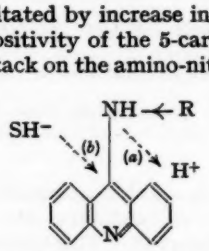
Those substituents R which render the group NHR labile are, with the exception of the p-nitrophenyl group, at once recognisable as increasing, by a $+ I$ inductive effect, the electron density on the 5-amino-nitrogen atom and thus increasing its basicity. Under alkaline conditions the 5-p-*nitroanilino*-derivative may, however, be expected to pass into the form :



That this is the case is to be inferred from its solubility in aqueous alkali and the deep red colour of the alkaline solution, properties not possessed by the m-*nitro*-isomer. Under the alkaline conditions of the reaction, therefore, it is apparent that a $+ I$ effect is present in the case of the p-nitro-derivative. The groupings which inhibit the reaction are, in contrast, $- I$ in their effect.

Bearing in mind the fact that the reaction takes place only in alkaline media, the simplest mechanism that can be postulated is an attack on the 5-carbon atom in the acridine nucleus by SH^- (process *a*) accompanied by the attachment of a proton to the amino-nitrogen atom (process *b*), though a mechanism involving $S^=$ ions cannot be excluded. In either case a $+ I$ effect by the group R will, as has been pointed out, increase the negativity of the nitrogen atom at position 5 and decrease the positivity of the 5-carbon atom. On the other hand a $- I$ effect by the group R will lower the basicity of the amino-nitrogen at 5 and increase the positivity of the 5-carbon atom.

The fact that the reaction is facilitated by increase in the negativity of the amino-nitrogen, instead of being diminished as the positivity of the 5-carbon atom is diminished, suggests that the rate-determining process is the attack on the amino-nitrogen by hydrions, process (*b*) :



Complete elucidation of the mechanism must await the results of kinetic experiments; nevertheless, support for the view put forward above has been obtained in a preliminary study of the reaction in the case of 5-p-*anisidinoacridine*. Thus we find that in aqueous alcoholic solution no reaction occurs in concentrated alkali (5 c.c. of 5N-sodium hydroxide + 20 c.c. of alcohol), where the hydrion concentration is very small. The rate is highest in the presence of solutions such as sodium carbonate and acetate, in which there is both sufficient alkalinity to provide an adequate concentration of SH^- and also a reservoir of the essential H^+ ion. Our results are summarised in the Table, which shows the yields of thioacridone obtained under different conditions of alkalinity. In each case 0·5 g. of 5-*p*-anisidinoacridine was dissolved in 20 c.c. of absolute alcohol, and 5 c.c. of a molar solution of the material shown were added; hydrogen sulphide was bubbled into the solution for 50 minutes at room temperature.

M-Solution added (5 c.c.).	Thioacridone precipitated after 50 mins. (%).	M-Solution added (5 c.c.).	Thioacridone precipitated after 50 mins. (%).
Ammonium chloride ...	0	Ammonium acetate ...	33
Ammonia	6	Sodium carbonate	40
Sodium hydroxide	12	Sodium acetate	40
Ammonium carbonate	13·7		

Experimental.

All but three of the acridine derivatives investigated are known substances and were prepared as described in the literature or supplied by Messrs. I.C.I., Blackley. Specimens of 5-(2-hydroxyethyl)-amino- and 5-(3-hydroxypropyl)amino-acridine were given by Dr. F. E. King.

5-p-*Anisidinoacridine.*—5-Chloroacridine (5 g.) was dissolved in phenol (30 g.) at 70°. *p*-Anisidine (4 g.) was added and the temperature maintained at 100°, with mechanical stirring, for 45 minutes. The mixture was cooled to 30° and poured into acetone at 0°. The solution was allowed to stand for 90 minutes, by which time the bright scarlet hydrochloride of 5-*p*-anisidinoacridine had been precipitated. The precipitate was collected and washed with acetone to free it from phenol. The hydrochloride was dissolved in water, the free *base* precipitated with N-sodium hydroxide, dried over calcium chloride, and recrystallised from light petroleum (b. p. 100—120°). Yield, 62%; m. p. 149° (uncorr.) (Found: C, 79·5; H, 5·34. $C_{20}H_{16}ON_2$ requires C, 80·0; H, 5·33%).

5-(m-*Nitroanilino*)*acridine.*—This *base* was prepared as above using 5-chloroacridine (10 g.) and *m*-nitroaniline (8 g.) in phenol (65 g.). Yield, 60%. Recrystallised from aqueous alcohol it had m. p. 177° (uncorr.) (Found: C, 72·8; H, 4·4. $C_{19}H_{13}O_2N_3$ requires C, 72·4; H, 4·2%).

5-(p-*Nitroanilino*)*acridine.*—This was prepared as above, using 5-chloroacridine (10 g.) and *p*-nitroaniline (8 g.) in phenol (65 g.). The hydrochloride was dissolved in water and N-sodium hydroxide added till the solution was just neutral to litmus. (Excess of alkali redissolves the base as the sodium salt.) Yield, 80%. Recrystallised from aqueous alcohol, the *base* formed yellow-orange needles, m. p. 221° (uncorr.) (Found: C, 71·9; H, 4·0; N, 13·1. $C_{19}H_{13}O_2N_3$ requires C, 72·4; H, 4·2; N, 13·3%).

Action of Hydrogen Sulphide on the 5-Aminoacridines.—Each reaction was examined by dissolving the 5-aminoacridine (0·5 g.) in absolute alcohol (20 c.c.), adding ammonia (*d* 0·88; 5 c.c.), and passing hydrogen sulphide through the solution until precipitation was complete. The solution first goes dark red owing to super-saturation with thioacridone, which is then suddenly precipitated. The yields varied between 75 and 90%.

R.	Yield of thioacridone (%).	R.	Yield of thioacridone (%).
Me	75	*p*-Nitroanilino-	20 (after 24 hours)
p-Anisidino-	90	5-Chloro-	90

The thioacridone obtained from these reactions recrystallised from methyl alcohol as dark red needles, m. p. 266° (uncorr.) (Found: C, 73·5; H, 4·29; N, 6·46. Calc. for $C_{13}H_9NS$: C, 73·9; H, 4·33; N, 6·63%).

2-Chloro-7-methoxythioacridone was formed in 90% yield from 2-chloro-5-(2-hydroxyethylamino)-7-methoxyacridine, mepacrine, and 2-chloro-5-(3-hydroxypropylamino)-7-methoxyacridine. It recrystallised from absolute alcohol as red-gold plates, m. p. 254° (uncorr.) (Found: C, 60·73; H, 3·87; N, 5·15; Cl, 13·1; S, 12·1. Calc. for $C_{14}H_{10}ONClS$: C, 61·0; H, 3·63; N, 5·08; Cl, 12·9; S, 11·61%).

Preparation of Thioacridone and p-*Anisidine from* 5-p-*Anisidinoacridine.*—*p*-Anisidinoacridine (15 g.) was dissolved in absolute alcohol (200 c.c.), and ammonia (*d* 0·88; 10 c.c.) added. Hydrogen sulphide was passed through the solution for 3 hours. Excess of alcohol was distilled off on a water-bath and the residue shaken with 5% sodium hydroxide solution (200 c.c.) to dissolve the thioacridone. This solution was extracted with ether (200 c.c.). The ether layer was dried ($CaCl_2$) and then the ether was evaporated off on a water-bath. The impure *p*-anisidine in the residue was not isolated but converted directly into the acetyl derivative, m. p. 127°. Yield, 23%. Thioacridone was obtained in 90% yield from the sodium hydroxide solution by acidifying and collecting the precipitate.

Dyson Perrins Laboratory, University of Oxford. [*Received, August 28th,* 1947.]

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234. *The Isolation of the Hitherto Unknown* anti-*Diazosulphonates in the Naphthalene Series, with Improved Preparations of* 2- *and* 4-*Nitro-and* 4-*Chloro*-1-*naphthylamine.*

By Herbert H. Hodgson and Donald Bailey.

Naphthalene-α- and -β-*anti*-diazosulphonates, which Hantzsch and Schmiedel failed to identify among the reaction products from diazotised α- and β-naphthylamine with alkali sulphites, have now been prepared, and in addition the *anti*-diazosulphonates from 4-nitro- and 4-chloro-1-naphthylamine have also been isolated. The corresponding four *syn*-diazosulphonates, which are really diazo-sulphites, decompose far more rapidly than the benzene analogues, and by a two-way mechanism, to give the corresponding symmetrical azo-compound on the one hand and the *anti*-diazosulphonate on the other. The order of *instability* of these naphthalene diazo-sulphites is 4-chloro > α- and β- > 4-nitro-, and can be accounted for on resonance grounds.

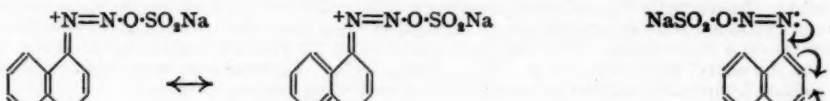
Hantzsch and Schmiedel (*Ber.*, 1897, **30**, 81) first prepared their so-called *syn*-sulphonates in the naphthalene series from α- and β-naphthylamine by the action of alkali sulphites on their diazonium salts. The *anti*-forms were not realised by these authors, because they believed that the *syn*-forms changed completely into 1 : 1′- and 2 : 2′-azonaphthalene respectively. This

formation of symmetrical azo- from diazo-compounds was subsequently the subject of a patent by Lange (D.R.-P., 78225) in which the reaction of a diazonium salt with an alkali sulphite was carried out in the presence of acetic acid.

Recently a paper by Hodgson and Marsden (*J.*, 1943, 470) has provided conclusive evidence that Hantzsch's stereochemical conception of *syn*- and *anti*-diazosulphonates in the benzene series was fundamentally wrong, and that the alleged *syn*-geometrical isomerides were actually diazosulphites, whereas the alleged *anti*-forms were the true diazosulphonates. Since Hodgson and Marsden (*loc. cit.*) found that the unstable sulphites (alleged *syn*-forms) could never be obtained pure but were always mixed with their relatively stable *anti*-sulphonate isomerides into which they passed rapidly, it appeared probable that the naphthalene analogues would behave in like manner, and that stable *anti*-diazosulphonates could be prepared in the naphthalene series also.

It is now shown that Hantzsch's alleged 1- and 2-naphthalene-*syn*-diazosulphonates experience a very rapid two-way conversion, *viz.*, (1) into the 1 : 1′- and 2 : 2′-azonaphthalene respectively, and (2) into the non-coupling 1- and 2-naphthalenediazosulphonates, so that the anti-*diazosulphonates* which Hantzsch failed to detect are actually found to possess a stable existence. These naphthalene-*anti*-diazosulphonates are red-brown substances, whereas the isomeric covalent sulphites (alleged *syn*-sulphonates) are yellow, and their presence and composition have been established by oxidation with bromine to diazonium salts which then couple with β-naphthol to give the corresponding 1- and 2-naphthaleneazo-β-naphthol. 4-Nitro-1-naphthalenediazonium chloride has also been found to give a more stable diazosulphite than the unsubstituted diazotised naphthylamines above, especially when treated with sodium sulphite in neutral solution, and this sulphite changes much more slowly (3 hours) than the corresponding unsubstituted naphthalenediazosulphites (a few seconds) into a mixture of 4 : 4′-dinitro-1 : 1′-azonaphthalene and *sodium 4-nitronaphthalene-1-diazosulphonate*. In like manner, 4-chloro-1-naphthylamine gives a mixture of 4 : 4′-dichloro-1 : 1′-azonaphthalene and *sodium 4-chloronaphthalene-1-diazosulphonate* in neutral solution, the transition from the sulphite being much the most rapid of the four diazonium salts examined.

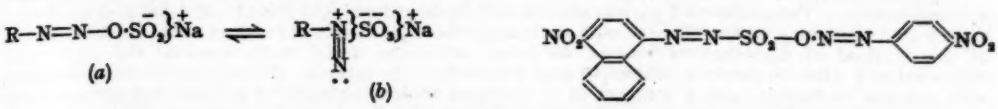
The sequence of instability of the above four diazosulphites is: 4-chloro- > unsubstituted > 4-nitro-, and an explanation based on resonance is suggested as follows. In the case of 4-nitronaphthalene-1-diazosulphite, the resonance of the nitro-group with the naphthalene nucleus will increase the number of resonance structures (10 for the α-acid, 9 for the β-unsubstituted compounds) to 20, while there will also be the two quinonoid resonance structures, bringing the



total to 22. This increase in the number of structures of the 4-nitronaphthalene-1-diazosulphite hybrid over the 1- and 2-naphthalenediazosulphites corresponds to an increase in resonance energy with a consequent increase in stability. In the case of the 4-chloronaphthalene-1-diazosulphite, however, there will still be the 10 structures of the unsubstituted compound, but the resonance effect of the chlorine atom will be opposed to that of the diazo-nitrogen, thereby reducing the contribution of those structures which involve a separation of charge. In consequence, the 4-chloronaphthalene-1-diazosulphite will be less stable than any of the other diazosulphites mentioned.

In aqueous solution these diazosulphites (*a*) are probably in equilibrium with their diazonium isomerides (*b*), since Hantzsch and Reddelien (" Die Diazo-Verbindungen ", 1921, p. 50) found that the aryl-*syn*-diazosulphonates afforded ions of the diazonium sulphite in solution, whence the diazo-form (*a*) subsequently isomerises to the *anti*-diazosulphonate (cf. Hodgson and Marsden, *loc. cit.*), and the diazonium form (*b*) decomposes to give the symmetrical azo-compound by the mechanism of Hodgson, Nicholson, and Turner (*J.*, 1944, 15) in which the diazo- and aryl radicals, R–$\dot{N}$=$\dot{N}$· and ·R, combine. The naphthalenediazosulphites decompose much more rapidly than their benzene analogues, with the consequence that the main product is the symmetrical azo-compound, whereas in the benzene series the main product is the *anti*-sulphonate. It was this rapidity of transformation to azo-compound that caused Hantzsch

and Schmiedel to miss finding the *anti*-diazosulphonates in the decompositions of naphthalene-1- and -2-diazosulphites (*syn*-sulphonates).



(*a*) (*b*)

Sodium 4-nitronaphthalene-1-*anti*-diazosulphonate readily reacts with diazotised *p*-nitroaniline to form a compound, as with the *anti*-diazosulphonates of the benzene series (cf. Hodgson and Marsden, *loc. cit.*), and this compound on reaction with alkaline β-naphthol gives a precipitate of *p*-nitrobenzeneazo-β-naphthol, leaving sodium naphthalene-1-*anti*-diazosulphonate in solution, from which it may be recovered intact or, after oxidation with bromine, as a diazonium salt which then couples with β-naphthol to give 4-nitronaphthalene-1 : 1′-azo-2′-naphthol. It follows, therefore, that the very stable N–S link of the original anti-structure remains intact during the coupling reaction. The corresponding reaction of diazotised *p*-nitroaniline with the sodium 4-nitronaphthalene-1-diazosulphite (Hantzsch's *syn*-compound), which was carried out as quickly as possible after the addition of sodium sulphite to 4-nitronaphthalene-1-diazonium chloride, afforded a solid which reacted with alkaline β-naphthol to give a mixed precipitate of 4-nitronaphthalene-1 : 1′-azo-2′-naphthol and *p*-nitrobenzeneazo-β-naphthol; the filtrate from this mixture when oxidised with bromine water also coupled with alkaline β-naphthol to give a mixture of the same azo-naphthols. These results, in contrast to the former unique data, indicate that the diazosulphite is changing to the diazosulphonate immediately after formation and in an exactly analogous manner to the phenomena obtained in the benzene series (cf. Hodgson and Marsden, *loc. cit.*).

An improved preparation of 4-chloro-1-naphthylamine, and a modified procedure for the separation of the 2- and 4-nitro-1-naphthylamines from the mixture given by the nitration of aceto-1-naphthalide, are described.

Experimental.

Action of Sodium Sulphite on Diazotised α-Naphthylamine.—A stirred hot solution of α-naphthylamine (3·6 g.) in hydrochloric acid (10 c.c., *d* 1·18) and water (10 c.c.) was chilled to 0°, ice (20 g.) added, and the fine suspension treated as rapidly as possible with one of sodium nitrite (2 g.) in water (10 c.c.), the mixture kept for 10 minutes, filtered, rapidly neutralised to Congo-red with calcium carbonate, and mixed immediately with a solution of crystallised sodium sulphite (6 g.) and sodium carbonate (1 g.) in water (25 c.c.). An orange-yellow precipitate of sodium naphthalene-1-diazosulphite (Hantzsch's *syn*-sulphonate) forthwith separated, and, if filtered off rapidly and washed at 0°, it coupled with alkaline β-naphthol to give 1-naphthaleneazo-β-naphthol (which gave a deep violet colour with concentrated sulphuric acid). When the mixture, however, continued to be stirred at 0°, it changed in a short time to a red-brown colour with evolution of nitrogen, and the solid portion then no longer reacted with alkaline β-naphthol. After the evolution of nitrogen had ceased, the mixture, which had been kept at 0° for *ca.* 10 minutes, was diluted with an equal volume of water and filtered; the precipitate, after being thoroughly washed with water until no more had dissolved, was 1 : 1′-azonaphthalene, and the filtrate, which did not couple with alkaline β-naphthol or ethyl-alcoholic β-naphthylamine, did so after being made just acid to Congo-red, freed from any excess nitrous acid with urea, and treated with bromine water until a slight excess was present as denoted by starch–potassium iodide paper, to give 1-naphthaleneazo-β-naphthol (0·65 g.), which crystallised from glacial acetic acid in red needles, m. p. and mixed m. p. with authentic specimen 229—230° (Meldola and Hanes, *J.*, 1894, **65**, 837, give m. p. 229—230°), and gave a violet colour with concentrated sulphuric acid. The 1 : 1′-azonaphthalene crystallised from glacial acetic acid in red needles, m. p. 198° (Nietzki and Goll, *Ber.*, 1885, **18**, 298, give m. p. 190°). The solution above thus contained, originally, the sodium naphthalene-*anti*-1-diazosulphonate, and another batch was evaporated to dryness on the water-bath, the residue extracted with hot ethanol, and the anti-*diazosulphonate* precipitated from the filtered extract by the addition of ether; the red-brown solid so obtained was purified by a repetition of the ethanol–ether treatment and could be recrystallised from water (Found : Na, 8·7. $C_{10}H_7O_3N_2SNa$ requires Na, 8·9%). When dissolved in water, the solution would not couple with alkaline β-naphthol but did so after treatment with bromine water as above.

Action of Sodium Sulphite on Diazotised β-Naphthylamine.—A hot solution of β-naphthylamine (3·6 g.) in hydrochloric acid (10 c.c., *d* 1·18) and water (30 c.c.) was chilled rapidly to 0°, and the finely divided precipitate of the hydrochloride was treated at 0° as quickly as possible with a solution of sodium nitrite (2 g.) in water (10 c.c.), and the mixture was then neutralised with calcium carbonate and treated with sodium sulphite as above. The immediate bright yellow precipitate (Hantzsch's *syn*-compound) changed in a few seconds to a fawn colour with a violent evolution of nitrogen. After the mixture had been kept at 0° for 5 minutes, it was stirred with an equal volume of water and filtered; the crude solid 2 : 2′-azonaphthalene crystallised from chloroform in dark red prisms, m. p. 204° (Hantzsch and Schmiedel, *loc. cit.*, give m. p. 204°). The filtrate, which did not couple with alkaline β-naphthol or with ethyl-alcoholic β-naphthylamine, did so after cautious addition of bromine water to give 2-naphthaleneazo-β-naphthol, which crystallised from glacial acetic acid in red-brown needles, m. p. and mixed m. p. with authentic specimen, 178—179° (Meldola and Hanes, *loc. cit.*., give m. p. 178—179°) and gave a

red-violet colour with concentrated sulphuric acid. The red-brown *sodium naphthalene-2-diazosulphonate* was obtained like its isomeride above (Found : Na, 8·7%).

Experiments with 4-*Nitro*-1-*naphthylamine.*—(a) *Sodium* 4-*nitronaphthalene*-1-*diazosulphite* (*Hantzsch's* syn-*sulphonate*). The amine (4·7 g.) was stirred with hydrochloric acid (10 c.c., *d*, 1·18) and ice (20 g.), and treated as rapidly as possible at 0° with sodium nitrite (2 g.) dissolved in water (10 c.c.) (the efficiency of this method of diazotisation was *ca.* 60% by nitrometer test), when most of the paste went into solution; after 30 minutes, water (10 c.c.) was added, the mixture filtered, the filtrate neutralised with calcium carbonate, and a solution of crystallised sodium sulphite (5 g.) and sodium carbonate (0·5 g.) in water (20 c.c.) added as rapidly as possible at 0°. The immediate orange-yellow precipitate of sodium 4-nitronaphthalene-1-diazosulphite, which then coupled with alkaline β-naphthol, gradually darkened to a red-brown solid during 3 hours with very gradual evolution of nitrogen, after which it no longer coupled with alkaline β-naphthol; it was then a mixture of sodium 4-nitronaphthalene-1-diazosulphonate and 4 : 4′-dinitro-1 : 1′-azonaphthalene, since it also gave a brilliant blue colour with concentrated sulphuric acid. The orange-yellow precipitate from a second experiment was completely soluble in water to form a solution which evolved sulphur dioxide when treated with mineral acids, decolorised iodine, and coupled with alkaline β-naphthol to give 4-nitronaphthalene-1 : 1′-azo-2′-naphthol which crystallised from nitrobenzene in dark purple needles, m. p. 262—263° (Hodgson, Nicholson, and Turner, *loc. cit.*, give m. p. 258°), and gave with concentrated sulphuric acid a purple colour, with alcoholic potassium hydroxide a violet colour, and with acetone followed by sodium hydroxide a scarlet colour, which changed to blue. This solution of the sodium 4-nitronaphthalene-1-diazosulphite, on standing, deposited 4 : 4′-dinitro-1 : 1′-azonaphthalene, which crystallised from nitrobenzene in red needles, m. p. 334° (Hodgson, Nicholson, and Turner, *loc. cit.*, give m. p. 334°), and the filtrate then coupled with alkaline β-naphthol only after cautious oxidation with bromine water to give 4-nitronaphthalene-1 : 1′-azo-2′-naphthol (identified as above), while it no longer behaved like the solution of a sulphite.

A solution of the sulphite prepared as above from 4-nitro-1-naphthylamine (4·7 g.) was stirred at 0° with a solution of *p*-nitrobenzenediazonium chloride (from 2 g. of *p*-nitroaniline) which had been neutralised with calcium carbonate, and the yellow precipitate which immediately formed was collected and washed with ice-water (1—1½ l.) until free from diazonium salt, as tested for by β-naphthol-coupling of the filtrate; it was then stirred into a solution of β-naphthol (2·5 g.) in 2% aqueous sodium hydroxide (slight excess) to give a mixture of 4-nitronaphthalene-1 : 1′-azo-2′-naphthol and *p*-nitrobenzeneazo-β-naphthol, m. p. 160°. The filtrate from the precipitate of azo-compounds was oxidised with bromine water and again coupled with alkaline β-naphthol to give a mixture, m. p. 172°, of the same azo-naphthols as above. This experiment indicated that the sodium 4-nitronaphthalene-1-diazosulphite above was mixed with the isomeric diazosulphonate as in the analogous benzene compounds (cf. Hodgson and Marsden, *loc. cit.*).

(b) *Sodium* 4-*nitronaphthalene*-1-*diazosulphonate.* A sample of the sodium 4-nitronaphthalene-1-diazosulphite was prepared as above and the mixture allowed to stand for 3 hours; the separated solids were then filtered off and stirred with water (200 c.c.), again filtered off, and the two filtrates combined. A red-brown solution of the *sodium* 4-*nitronaphthalene*-1-*diazosulphonate* was thus obtained which did not couple with alkaline β-naphthol but did so after oxidation with bromine water to give 4-nitronaphthalene-1 : 1′-azo-2′-naphthol (identified as above). The sodium salt was also isolated as for the analogous naphthalenediazosulphonates as an orange-red solid (Found : Na, 7·4. $C_{10}H_6O_5N_2SNa$ requires Na, 7·6%).

Coupling of Sodium 4-*Nitronaphthalene*-1-*diazosulphonate.*—(a) *With diazotised aniline.* The sodium salt (from 4·7 g. of 4-nitro-1-naphthylamine) was made into a paste with water, and then diluted to a fine suspension which was then stirred with a solution of benzenediazonium chloride that had been previously neutralised with calcium carbonate; rapid decomposition immediately ensued with formation of a black tar.

(b) *With diazotised* p-*nitroaniline* (*from* 2 *g.*) *made neutral with calcium carbonate.* A yellow precipitate resulted, which was filtered off, washed with ice-water (1—1½ l.) until free from diazonium salt, and then stirred into a solution of β-naphthol (5 g.) in 2% aqueous sodium hydroxide (slight excess); *p*-nitrobenzeneazo-β-naphthol *only* separated and was filtered off; it crystallised from glacial acetic acid, m. p. and mixed m. p. with authentic specimen, 250°. The filtrate above was just acidified at 0° (ice), the precipitated β-naphthol filtered off, and the filtrate oxidised carefully with bromine water; it then again coupled with alkaline β-naphthol, this time to give 4-nitronaphthalene-1 : 1′-azo-2′-naphthol which crystallised from nitrobenzene in dark purple needles, m. p. and mixed m. p. with authentic specimen, 262—263°.

Experiments with 4-*Chloro*-1-*naphthylamine.*—The amine (2·2 g.) was stirred with hydrochloric acid (5 c.c., *d* 1·18), water (10 c.c.), and ice (10 g.), diazotised by rapid addition of sodium nitrite (1 g.) dissolved in water (5 c.c.), and the mixture kept for 30 mins., filtered, neutralised with calcium carbonate, and finally treated rapidly with a solution of crystallised sodium sulphite (3 g.) and sodium carbonate (0·25 g.) in water (10 c.c.). The bright yellow precipitate which separated immediately did not change colour on keeping, although there was a brisk evolution of nitrogen, and it appeared to be a mixture 4 : 4′-dichloro-1 : 1′-azonaphthalene and sodium 4-chloronaphthalene-1-diazosulphonate (Hantzsch's *anti*-compound) since there was no coupling with alkaline β-naphthol. After removal and stirring of the solid with water, the mixture was filtered. The filtrate coupled with alkaline β-naphthol only after oxidation with bromine water, when 4-*chloronaphthalene*-1 : 1′-*azo*-2′-*naphthol* was precipitated; this crystallised from nitrobenzene in dark red needles, m. p. 218—220° (Found : N, 8·6. $C_{20}H_{12}ON_2Cl$ requires N, 8·4%). The yellow solid *sodium* 4-*chloronaphthalene*-1-*diazosulphonate* was obtained as above (Found : Na, 7·6. $C_{10}H_6O_3N_2ClSNa$ requires Na, 7·86%).

4-*Chloro*-1-*naphthylamine.*—A solution of aceto-1-naphthalide (18·5 g.) in glacial acetic acid (60 c.c.) and hydrochloric acid (23 c.c., *d* 1·18) was cooled below 20° and treated gradually with a solution of sodium chlorate (6 g.) in water (25 c.c.), the temperature being kept below 50°. 4-Chloroaceto-1-

naphthalide partly separated, and the precipitation was completed by addition of water; the orange-yellow product (20 g.) was then collected and dried; it was hydrolysed by heating it with ethanol (100 c.c.) and 50% aqueous sulphuric acid (100 c.c.) for an hour, after which the crude sulphate of 4-chloro-1-naphthylamine was precipitated by adding the mixture to water, and recrystallised from dilute sulphuric acid (1 part acid of *d* 1·84 and 2 parts water). On basification of the sulphate crystals with ammonia and subsequent crystallisation from aqueous ethanol (charcoal), the 4-chloro-1-naphthylamine was obtained in very pale pink, almost colourless needles, m. p. 98° (Reverdin and Crépieux, *Ber.*, 1900, **33**, 682, give m. p. 98°).

Alternative Separation of 2- *and* 4-*Nitro*-1-*naphthylamine.*—After hydrolysis of the mixture of 2- and 4-nitroaceto-1-naphthalides obtained by the mononitration of aceto-1-naphthalide (for details see Hodgson and Walker, *J.*, 1933, 1205), the solid obtained by dilution with water of the hydrolysis mixture was removed and boiled with hydrochloric acid (500 c.c., *d* 1·18) and water (1 l.), and the mixture filtered; the dissolved 4-nitro-1-naphthylamine, m. p. 187—189°, separated from the filtrate on cooling, and on recrystallisation from glacial acetic acid (*ca.* 13 c.c. per g.) afforded yellow-brown needles, m. p. 193—194° (Hodgson and Walker, *loc. cit.*, give m. p. 193—194°). Further extractions with dilute hydrochloric acid as above gave decreasing amounts of a less pure amine, the m. p. of which finally reached 130—150°. The insoluble residue, which was mainly the 2-nitro-1-naphthylamine, was dissolved in glacial acetic acid (10 c.c. per g.), the solution filtered, and the base precipitated as sulphate by addition of sulphuric acid (5 c.c. per 100 c.c. solution); this was removed, washed with glacial acetic acid, and basified by water (m. p. 138—140°); it crystallised from glacial acetic acid or 90% formic acid in orange-yellow prisms, m. p. 143—144° (Hodgson and Walker, *loc. cit.*, give m. p. 143—144°). Alternatively, the material of m. p. 138—140° was dissolved in boiling dilute sulphuric acid (1 part acid of *d* 1·84 and 2 parts water), and the solution filtered and diluted with water until precipitation was complete; this gave orange crystals of 2-nitro-1-naphthylamine, m. p. 143—144°.

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TECHNICAL COLLEGES OF HUDDERSFIELD AND HALIFAX. [*Received, August 29th*, 1947.]

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235. *The Heats of Formation of the* —N=N— *and* >C=N— *Bonds.*

By G. E. COATES and L. E. SUTTON.

The apparent heats of formation of bonds, or bond energy terms, are still very useful data, notwithstanding that the technical difficulties attending their derivation from combustion data and their theoretical limitations have recently been emphasised.

The terms for the N=N and the C=N bond have been obtained by burning azo*iso*propane and *n*-butyl*iso*butylideneamine (Q_v = 1053·$_4$ and 1295·$_4$ kcals./mole respectively). On the basis of graphite ⟶ carbon gas (^3P) − 126·3 kcals./mole and $N_2(g)$ ⟶ 2N(g) − 170·2 kcals./mole, these bond terms are 63·1 and 106.

Q_v values for benzylideneanil (1639·$_3$), dibenzylidene-ethylenediamine (2116·$_3$), dibenzylideneazine (1808·$_1$), and diphenylbutadiene (2023·$_1$) have also been measured. From resonance energies in these and related compounds it is concluded that the bond energy term for C=N may vary from 96 to 106.

The chemical consequences of this variation and of that in the C=O bond are illustrated. The relevance of the new values to the polymerisation of multiple-bonded substances, and to the resonance energy in polyene systems is discussed.

From available data it is tentatively concluded that the π bonds in C=C, N=N, and C=N have much the same strength, though the σ bonds vary greatly. In triple bonds there appears to be a drastic reorganisation resulting in a regular change of strength from C≡C, through C≡N, to N≡N.

GREAT importance attaches to the amount of energy evolved when a bond in a molecule is formed. Direct methods of measuring such values in polyatomic molecules have been developed, *e.g.*, spectrographic methods (see review by Samuel, *Rev. Mod. Physics*, 1946, **18**, 103), electron-impact methods (Smyth, *ibid.*, 1931, **3**, 347; Smith, *Physical Rev.*, 1937, **51**, 263; Hipple, *ibid.*, 1938, **53**, 530; Stevenson, *J. Chem. Physics*, 1942, **10**, 291), and the identification of the heats of activation of certain thermal reactions with the energy necessary to break a particular bond (Butler and Polanyi, *Trans. Faraday Soc.*, 1943, **39**, 19; Andersen and Kistiakowsky, *J. Chem. Physics*, 1943, **11**, 6; Kistiakowsky and Van Artsdalen, *ibid.*, 1944, **12**, 469; Andersen and Van Artsdalen, *ibid.*, p. 479; Andersen, Kistiakowsky, and Van Artsdalen, *ibid.*, 1942, **10**, 305).

These methods are still not of wide application, however, and most values have been obtained by analysing the heats of forming a whole molecule from its constituent atoms, on the assumption that this gross energy is the sum of the heats of forming the individual bonds (Sidgwick and Bowen, *Ann. Reports*, 1931, **28**, 386; Sidgwick "The Covalent Link in Chemistry", Cornell, 1933, Chap. IV; Pauling and Yost, *Proc. Nat. Acad. Sci.*, 1932, **18**, 414; Pauling, "The Nature of the Chemical Bond", Cornell, 1940, Chap. IV). We have in this way sought to obtain values for the C=N and the N=N bond.

The difficulties attending the indirect thermochemical method are several. The primary

assumption implies that all the bonds are strictly " localised " or independent; and if this is not so, the method fails, or at least runs into complications. The discrepancy between the actual values for the heats of forming certain molecules and those predicted on the assumption of additivity have indeed been used to detect and to measure the effects of particular kinds of non-localisation, especially that termed " resonance " (Pauling, *loc. cit.*; cf. Lennard-Jones and Coulson, *Trans. Faraday Soc.*, **1939**, **35**, 811); but other kinds are possible, *e.g.*, the overlap of bonding functions of adjacent or neighbouring bonds, hyperconjugation, steric repulsion, dipolar interaction, changes of bonding orbital; and the sorting out of these contributions has not been carried very far. Some deviations from additivity of up to 5—6 kcals./mole are observed in the paraffins, olefins, and alcohols (Rossini, *J. Res. Nat. Bur. Stand.*, **1934**, **13**, 21; Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Amer. Chem. Soc.*, **1935**, **57**, 876; Rossini, *J. Res. Nat. Bur. Stand.*, **1934**, **13**, 189) in which no " resonance " of the above-mentioned kind is to be expected.

Furthermore, the true heat of breaking a bond is a function not only of the molecule in which it is, but also of the fragments into which this is broken. If one or more radicals are formed, these may be stabilised or destabilised by changes in resonance energy, in bonding orbitals, or in steric energy.* This will cause the true value to differ from the average value which the analysis gives. There may be variations of heats of formation which compensate and so produce no obvious departure from additivity in the molecule as a whole. The actual heat of formation or dissociation of a particular bond cannot, therefore, be accurately identified with the average value of forming it from atoms, which is all that the thermochemical analysis can give. The distinction may be recognised by using different names for them; the energy for a particular bond, such as would be measured by a direct method, may be called the *bond energy*, while the thermochemical average may be called the *bond energy term* (Butler and Polanyi, *loc. cit.*). Long and Norrish (*Proc. Roy. Soc.*, **1946**, *A*, **187**, 337) and Walsh (*Trans. Faraday Soc.*, **1947**, **43**, 60) use " dissociation energy ", and " bond energy " or " bond heat of formation " respectively.

There are other difficulties too. One of the most serious is the continuing uncertainty about the heats of atomising some of the elements from their standard states, especially carbon and nitrogen (see later). The heat of promotion of carbon from its ^3P ground state into that in which it is ready to form four valencies (often said to be the ^5S state) is also very uncertain; and indeed the relevance of this energy to that of rupturing the first of four linkages formed by carbon (as in $CH_4 \longrightarrow CH_3 + H$) is not clear. If there is no demotion during rupture, this promotion energy, supposing it can be determined, gives the correct basis for making the bond energy terms approximately equal to the bond energies; but if there is any such demotion, it obviously does not. As a result of these uncertainties, the nominal bond energy terms may be very different from the true bond energies.

A further difficulty is that in general the thermochemical data cannot be corrected to absolute zero, but are reduced to some room temperature (18°, 20°, or 25°), so that the bond energy terms may be affected by the thermal energy of bond stretchings and bendings and group rotations.

The fraction which such a contribution makes to bond energy is small; and its importance in a discussion of absolute bond strengths would be trivial. In some hydrocarbons where it can be calculated, it is only about 0·5 kcal. per bond (Aston, *Chem. Reviews*, **1940**, **27**, 59). Dewar has, however, suggested (*Trans. Faraday Soc.*, **1946**, **42**, 767) that the total of the contributions may have an important effect upon the resonance energies as calculated by subtracting the sum of the bond energy terms for a normal structure from the observed heat of formation, and that this is the cause of the discrepancies between values so calculated and those derived from hydrogenation data (Dolliver, Gresham, Kistiakowsky, and Vaughan, *J. Amer. Chem. Soc.*, **1937**, **59**, 831). This discrepancy proves to be caused by failure to use the appropriate value for H_a (C=C) (Cottrell and Sutton, *J. Chem. Physics*, **1947**, **15**, 685). Kistiakowsky and his co-workers found that the heat of hydrogenation of a C=C bond increases as more alkyl groups are attacked, and they therefore used a value from *cyclo*hexene in deriving the benzene resonance energy. This was not done in calculations from combustion data: but when appropriate values from normal, or branched-chain olefins are used (see later), the discrepancies disappear. This substitution effect is almost as great at 0° K., and appears therefore to arise from some

* Attempts to correlate bond energies and stretching force-constants (see, *e.g.*, Linnett, *Trans. Faraday Soc.*, **1945**, **41**, 223) are limited by the fact that the latter relate to quite small perturbations of the initial state, so the effect on them of such changes in the final states is uncertain, and may be small. The same may be true of bond lengths and electric dipole moments. All these characteristics do, however, afford an indication of whether an anomalous bond energy arises from a change in the initial or in the final state.

bonding change, though until zero-point vibrational energies are eliminated this is not certain (see Cottrell, *J.*, in the press).

Fortunately, for the calculation of differences in processes not involving a change in the total number of bonds, *e.g.*, heats of reaction, or resonance energies as differences between actual and additive heats of formation, bond energy terms are adequate, and the difficulties concerning heats of atomisation are irrelevant; but for the discussion of reaction velocity in relation to absolute bond strengths, or in the comparison of the strengths of different bonds, bond energies are necessary. Our primary aim was to obtain information for the first purpose.

The only compounds containing an N=N bond which are free from extra resonance appear to be the azo-paraffins. Azomethane is a gas at room temperature and is not suited to the combustion technique used in the present work, namely bomb calorimetry. Azo*iso*propane boils at 88·6° and was chosen instead. A compound containing the smallest possible number of bonds is, other things being equal, the best for this purpose, since the unknown bond energy is then a larger part of the total heat of formation. Azobenzene has the advantage of being readily obtainable and easily purified; but it is likely to have extra resonance. Published data for it are discussed later.

Compounds not stabilised by extra resonance and containing a C=N bond are not at all common. The only possibilities appeared to be some azomethines which result from the condensation between primary aliphatic amines and some higher aliphatic aldehydes. Lower aliphatic aldehydes give more complex, often polymerised products with amines. *n*-Butyl-*iso*butylideneamine ($CH_3 \cdot [CH_2]_3 \cdot N{:}CH \cdot CHMe_2$) was chosen for the present purpose. The more readily accessible aromatic azomethines are of course likely to be stabilised by extra resonance; and by using the C=N value from the above compound we may expect to determine this. Combustion data were, therefore, obtained for benzylideneanil and dibenzylidene-ethylenediamine. In order to obtain further data, for related carbon and nitrogen systems, dibenzylideneazine ($CHPh{:}N \cdot N{:}CHPh$) and diphenylbutadiene were also measured.

Heats of Atomisation.—The dissociation energy of hydrogen ($H_a = 102{\cdot}48 \pm 0{\cdot}02$ kcals./mole at 0° K., for nH_2, Beutler, *Z. physikal. Chem.*, 1935, *B*, **29**, 315) and of oxygen ($H_a = 117{\cdot}0$ kcals./mole at 0° K., Herzberg, " Molecular Spectra and Molecular Structure ", New York, 1939, p. 491) are known with a fair degree of certainty.

That of nitrogen, and the heat of atomising graphite are still uncertain. The choice appears to lie between high values for both, if the importance of the non-crossing rule be accepted (Gaydon and Penney, *Proc. Roy. Soc.*, 1945, *A*, **183**, 374), or low ones for both if the arguments of Herzberg (*Chem. Reviews*, 1937, **20**, 145; *J. Chem. Physics*, 1942, **10**, 306) and of van der Ziel (*Physica*, Eindhoven, 1937, **4**, 373) be preferred. Both possible values, in each case, are accurately known, but the choice between them is a matter of dispute (cf. Long and Norrish, *loc. cit.*; Valatin, *J. Chem. Physics*, 1946, **14**, 568). We shall not try to resolve this difficulty; so where absolute values are discussed we shall quote values on both bases, calling the lower ones the A series, and the higher ones the B series.

The heats of atomisation (in kcals./mole) which we shall take are given in Table I.

TABLE I.

		H_a (0° K.).	H_a (293·1° K.).
Carbon (^3P from graphite)	A	125·1	126·3
	B	170·4	171·6
Hydrogen		51·24	51·71
Oxygen		58·5	58·9
Nitrogen	A	84·6	85·1
	B	112·5	113·0

In all the values at 20° the atomic gases are assumed to be perfect. The carbon A values are based on 9·14 e.v. for H_a(CO) at 0° K. to give ^3P carbon (Herzberg, *loc. cit.*); the B values on 11·11 e.v. (Gaydon and Penney, *loc. cit.*); $-\Delta H_0$ for C(graphite) + 1/2 $O_2 \longrightarrow$ CO (gas) is taken as 27·2 kcals./mole (Rossini, *J. Res. Nat. Bur. Stand.*, 1939, **22**, 407); the value of $H_{293{\cdot}1} - H_0$ taken for graphite (0·25 kcal./g.-atom) is that given by Rossini and Jessup (*ibid.*, 1938, **21**, 491; cf. Pitzer, *Chem. Reviews*, 1940, **27**, 53).

The values of $H_{293{\cdot}1} - H_0$ for hydrogen and oxygen are those given by Pitzer (*loc. cit.*); that for nitrogen is from Giauque and Clayton (*J. Amer. Chem. Soc.*, 1933, **55**, 4885). The A values for nitrogen are based on 7·38 e.v. for H_a (N_2) at 0° K. (van der Ziel, *loc. cit.*) and the B values on 9·764 e.v. (Gaydon and Penney, *loc. cit.*).

Experimental.

Preparation of Materials.—Azo*iso*propane was prepared by the method of Lochte, Noyes, and Bailey (*J. Amer. Chem. Soc.*, 1922, **44**, 2556); b. p. 88·6° ± 0·1° (corr.). *n*-Butyl*iso*butylideneamine, prepared by the condensation of purified *iso*butaldehyde and *n*-butylamine, was dried over potassium carbonate and twice fractionated, the fraction of constant b. p. 142·7° ± 0·1° (corr.)/755 mm. being taken for combustions (Found: N, 11·13. Calc.: N, 11·02%). Since this work was done a series of other similar compounds has been described by Campbell, Sommers, and Campbell (*ibid.*, 1944, **66**, 82).

Benzylideneanil was prepared as described in *Org. Synth.*, Coll. Vol. I, p. 73, and was crystallised three times from alcohol, m. p. 52° ± 0·2° (corr.). Before use it was dried in a vacuum. Dibenzylidene-ethylenediamine, from benzaldehyde and ethylenediamine (Mason, *Ber.*, 1887, **20**, 267), kindly supplied by British Celanese Ltd., was crystallised from alcohol; m. p. 54° ± 0·2° (corr.).

Dibenylideneazine and diphenylbutadiene were prepared according to *Org. Synth.*, Coll. Vol. II, p. 395 and p. 229, respectively; m. p.s 93° ± 0·3° and 153° ± 0·3°.

Benzoic, hippuric, and salicylic acids, for use as calorimetric standards, were each thrice crystallised from distilled water, and dried in a vacuum over concentrated sulphuric acid, and then in a vacuum over phosphoric oxide. Gelatine capsules for the combustion of liquids were kept all together in a closed bottle for several months before use, so that they all attained the same water content.

Calorimetric Method.—The Griffin–Sutton bomb calorimeter (*J. Sci. Instr.*, 1933, **10**, 286) was used in this work and the normal procedure for measuring heats of combustion was followed. Temperatures were measured with a mercury thermometer, reading to 0·01°, which had been calibrated at the N.P.L. The water equivalent was determined by means of eight combustions of benzoic acid (R.M.S. error 0·1%) [heat of combustion = q = 6·319 kcals.$_{15}$/g. (vac.), First Report of the Permanent Thermochemical Commission, 1933, p. 2], and checked by eight combustions of hippuric acid and three of salicylic acid. The measured heat of combustion of hippuric acid was 5·637 ± 0·013 kcals/g., which is 0·15% greater than the accurate value 5·628$_8$ found by Huffmann (*J. Amer. Chem. Soc.*, 1938, **60**, 1171). The heat of combustion of salicylic acid, q = 5·240 ± 0·015 kcals./g., was 0·10% greater than the value given by Keffler (*J. Physical Chem.*, 1929, **33**, 37). The bomb was filled with oxygen at 25 atm., but heats of combustion were corrected to atmospheric pressure by the method of Washburn (*Bur. Stand. J. Res.*, 1933, **10**, 525). The vacuum correction was applied to all weights. The random error is estimated to be not more than ± 0·25%, a degree of accuracy which is poor in comparison with that attained by modern precision technique, but sufficient for the purpose of obtaining bond energies in view of the numerous other errors which arise.

Results.

(All energy terms are given in kcals./mole.) *Azoisopropane.* From five combustions, Q, the heat of combustion per mole, was 1053·$_4$ ± 0·3 kcals. *n-Butylisobutylideneamine.* Five combustions, Q = 1295 ± 2·4. *Benzylideneanil.* Five combustions, Q = 1639 ± 1·7. *Dibenzylidene-ethylenediamine.* Four combustions, Q = 2116·5 ± 2·0. *Dibenzylideneazine.* Five combustions, Q = 1808·0 ± 3·1. *Diphenylbutadiene.* Five combustions, Q = 2023·0 ± 1·6. The following approximate heats of vaporisation and sublimation, obtained by means of Trouton's rule, were corrected to 20° (latent heats of fusion were assumed to be 4): azo*iso*propane, $L_v^{20°}$ = 8·5 ± 0·5; *n*-butyl*iso*butylideneamine, 10 ± 0·5; benzylideneanil, $L_s^{20°}$ = 20·5 ± 0·5; dibenzylidene-ethylenediamine, 22 ± 0·5; dibenzylideneazine, 22·3 ± 0·5; diphenylbutadiene, 22·3 ± 0·5.

Heats of formation were calculated with the use of Rossini's data for the heats of combustion of graphite and hydrogen (*loc. cit.*), *viz.*, C(graphite) + O_2 = CO_2, ΔH_{18}° = − 94·03; $H_2 + 1/2\ O_2$ = $H_2O_{(l.)}$, ΔH_{18}° = − 68·32. Using these values and the heats of atomisation given in Table I, the atomic heat of formation (H_a) at 20° of a gaseous compound $C_aH_bO_cN_d$ is related to its (positive) heat of combustion Q by

$$-H_a\,(\mathrm{A}) = Q + L_{v(s.)}^{20°} - (126{\cdot}3 + 94{\cdot}0)a - (51{\cdot}71 + 34{\cdot}16)b - 58{\cdot}9c - 85{\cdot}1d$$

and

$$-H_a\,(\mathrm{B}) = Q + L_{v(s.)}^{20°} - (171{\cdot}6 + 94{\cdot}0)a - (51{\cdot}71 + 34{\cdot}16)b - 58{\cdot}9c - 113{\cdot}0d$$

The errors in these standard values are so much less than those in Q and L_v that they have no appreciable effect upon the uncertainty in H_a.

In these equations, bond energies and atomic heats formation derived from series A in Table I are labelled (A), and those from series B are labelled (B). In the following calculations A values will be used: B values will be given later.* Hence the atomic heats of formation (A) of the compounds examined are: Azo*iso*propane, H_a = 1632 ± 0·5; *n*-butyl*iso*butylideneamine, 2002 ± 2·6; benzylideneanil, 2234 ± 2·0; dibenzylidene-ethylenediamine, 2930 ± 2·2; dibenylideneazine, 2454·5 ± 3·3; diphenylbutadiene, 2681·5 ± 1·8.

The numbers represent, in the language of the additivity rule, the sum of the energy terms of all the bonds in the molecule and any extra resonance energy. In order to derive the energy terms for the C=N and N=N bonds, it is, therefore, necessary to decide those for the other bonds, *viz.*, C–C, C=C, C–H, and C–N.

In the paraffin series the increase in Q per CH_2 group becomes constant at 157·4 kcals./mole† at pentane (g.) and hexane (g.) (Prosen and Rossini, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 263). Hence, from the expression for H_a, the energy of one C–C bond and of two C–H bonds is 234·6 kcals./mole. By combining this with the heats of formation of the individual paraffins, a series of values for H_a(C–C) and

* The formula for conversion from A into B values is $H_a(\mathrm{B}) = H_a(\mathrm{A}) + 11{\cdot}3x + 9{\cdot}3y$, where x and y are the numbers of ends of single bonds attached to carbon or nitrogen respectively for the bond in question.

† These and other values given by Rossini are actually for 298° K., not 293°: the difference is negligible.

H_a(C–H) are obtained which become steady at hexane (practically at pentane) at 60·3 for C–C and 87·15 for C–H [cf. 59·6 and 87·8, respectively, used by Pauling, *op. cit.*, p. 53, corrected to 126·3 for H_a(C, graphite)]. These values will be taken because the hydrocarbon radicals in the compounds we have examined contain three or more carbon atoms.

By combining these values with the heats of forming *n*-1-olefins (Prosen and Rossini, *J. Res. Nat. Bur. Stand.*, 1946, **36**, 269) a series of values for the C=C bond may be obtained which becomes steady at *n*-pent-1-ene at 101·2 kcals./mole which will be adopted (Pauling's value, *op. cit.*, p. 131, is 102).

For evaluating the resonance energy in benzene a different value is, however, appropriate, namely, that from *cis*-hex-3-ene, which is 102·9 kcals./mole; while for substituted benzenes, with carbon replacing hydrogen, each substitution is accompanied by a change in one double-bond value to 106·4, that taken from 2-methylbut-2-ene (cf. Cottrell and Sutton, *loc. cit.*).

To obtain the C–N bond energy term from the heats of combustion of amines (Kharasch, *J. Res. Nat. Bur. Stand.*, 1929, **2**, 359) it is in most cases necessary to know the N–H bond energy term; this will be taken as 83·7 kcals./mole from data on ammonia (Pauling, *op. cit.*; *loc. cit.*). The following are the C–N terms calculated by using the above values for the C–C, C–H and N–H terms: Methylamine 47·8, ethylamine 48·1, *n*-propylamine 48·4, butylamines 54·8, *iso*amylamine 55·5, *n*-hexylamine 55·9, *n*-heptylamine 56·4, dimethylamine 45·4, diethylamine 51·0, di*iso*butylamine 53·4, di*iso*amylamine 54·8, triethylamine 53·1, tri*iso*butylamine 55·1, tri*iso*amylamine 50·4. The average value 52·5 will be used (Pauling gives 49·0, corrected to our value for H_a of C), but it cannot be regarded as very definite.

From the above data we calculate H_a for the –N=N– bond to be 65·7 kcals./mole in azo*iso*propane, without correcting for the possible difference in the *iso*- and *n*-propyl radicals: assuming the correction to be the same whether nitrogen or carbon is attached, the value is 63·1. The value of the >C=N bond in *n*-butyl*iso*butylideneamine is 106·1 kcals./mole or 104·8 corrected for the "iso-effect" (Pauling, *loc. cit.*, estimates this as 96).

For comparison with the –N=N– and the >C=N– bond, the energies of >N–N< and of –C≡N have been calculated from the heat of formation of hydrazine ($-\Delta H^{\circ}_{25^{\circ}\text{ c. (g.)}} = 22{\cdot}250$; Corruccini, Hughes, and Gilbert, *J. Amer. Chem. Soc.*, 1939, **61**, 2639) and the heat of combustion of methyl cyanide (Rossini and Bichowsky, "Thermochemistry of Chemical Substances", New York, 1936) as 20·4 and 151·6 kcals./mole, respectively. The C–O and C≡C bond energy terms have similarly been calculated from the data of Rossini (*J. Res. Nat. Bur. Stand.*, 1934, **13**, 189; *Amer. Pet. Inst. Rep.*, 1945) as 74·1 and 132·7 kcals./mole, respectively, the latter being the steady value from 1-ynes; Pauling gives 70·65 and 126·0, respectively.

From data in Bichowsky and Rossini's and Kharasch's compilations the value for H_a(C=O) is calculated as 143 kcals./mole in formaldehyde, 153·4 in acetaldehyde, 153·7 in propaldehyde, 156 in acetone, and 155 in methyl propyl ketone (cf. Pauling, *loc. cit.*).

The values are collected in Table II (p. 1193), which also includes values for O–O and O=O. The former is derived from water and hydrogen peroxide; the latter is Pauling's estimate of the value for the $^1\Delta$ state of molecular oxygen.

It may be re-emphasised that these are bond energy terms, *i.e.*, values chosen primarily to make possible the calculation of molecular heats of formation by simple addition. Different values for N–N and O–O have been proposed, *e.g.*, by Skinner (*Trans. Faraday Soc.*, 1945, **41**, 645; see, however, Glockler and Matlack, *J. Chem. Physics*, 1946, **14**, 504), by Walsh (*Trans. Faraday Soc.*, 1947, **43**, 60), and for O–O by Bolland and Gee (*ibid.*, 1946, **42**, 244); but these are bond energies, more appropriate in discussions of absolute bond characteristics, but not necessarily suitable for the above purpose. From the nature of the process used in deriving bond energy terms, which is to take as primary certain bonds which occur very often, *e.g.*, C–H and C–C, and to treat others as secondary or even tertiary, a variation in the heat of formation of the molecule as a whole may correspond to large variations in the term for one particular secondary bond. Thus, we see that the C=O term varies from 143 to 156; but, according to Skinner (*loc. cit.*), the change in the C=O bond itself is small, the alterations being mainly in the other bonds. This must be remembered if bond energy terms are used, *faute de mieux*, for bond energies. Artificial as the convention may seem, it is the simplest one.

Discussion.

From the energy terms found for the –N=N– and the >C=N– bond we may now attempt to calculate resonance energies in some conjugated systems containing nitrogen.

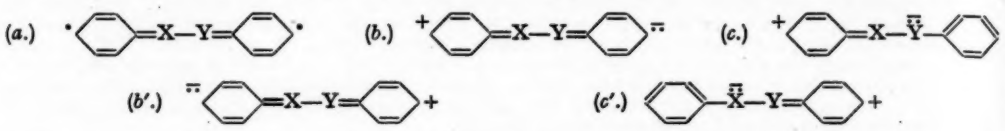
Corruccini and Gilbert (*J. Amer. Chem. Soc.*, 1939, **61**, 2925) and Richardson and Parks (*ibid.*, p. 3543) give values for the heats of combustion of *trans*-azobenzene and of stilbene, respectively. From them and from our own value for benzylideneanil the total resonance energies may be calculated. These may be uncorrected either for the benzene rings being substituted (p. 1188) or for the "*iso*-effects" in the –N=N– and the >C=N bond (see above); or corrections may be made for both, it being assumed where appropriate that the effect of substitution of hydrogen by nitrogen is the same as that by carbon.

Subtraction of the resonance energy in the benzene rings (36 kcals./mole in each) then gives that peculiar to the molecule as a whole. These values are collected below:

	Azobenzene.	Benzylideneanil.	Stilbene.
Uncorr.	12·7	5·1	15·7
Corr.	8·3	−0·6	8·7

There is no apparent theoretical reason why the value for benzylideneanil should

be so low or so different from the others. Using the electron-pair-bond description, we should say that the main structures, besides the " normal " ones, contributing to the overall resonance are * (*a*), (*b*), (*b'*), (*c*), and (*c'*) together with corresponding ortho-structures.



The resonance energies will depend inversely upon the differences of energy between them and the " normal " structure. Now the differences for type (*a*) can be calculated from the bond energy terms given, and are 23·2, 42·6, and 42·6 kcals./mole for azobenzene, benzylideneanil, and stilbene, respectively. The corresponding ones for structures of type (*b*) would bear the same relation among themselves as would those above, though they would differ from the latter by the energy needed for the electron transfer common to all. The structures of type (*c*) and (*c'*) would show different relations. Nitrogen having a greater electronegativity than carbon, these energy differences would rise in the order azobenzene < benzylideneanil < stilbene. The resonance energy should therefore be, on the above arguments, greatest in azobenzene, least in stilbene, and intermediate in benzylideneanil; and not in that supposedly observed, *viz.*, stilbene ⩾ azobenzene > benzylideneanil.

The corrected particular resonance energies in dibenzylideneazine and in diphenylbutadiene are 4·4 and 23·6, respectively. Here also the difference is far larger than would be expected.

There is a further anomaly. If the above bond energy terms are used to calculate the total resonance energy in dibenzylidene-ethylenediamine, the value 58·7 (uncorr.) to 54·3 (corr.) kcals./mole is obtained; but this is 13·3 to 17·7 kcals./mole *less* than that in the two benzene rings alone. This result is highly improbable.

Yet again, these bond values give also a resonance energy of only 27·2 kcals./mole in pyridine, compared with 36 in benzene, which also seems unlikely.

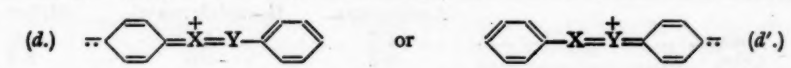
It is probably significant that if a value of 96 kcals./mole be used as the energy term for the C=N bond, instead of the 106 used above, then (i) the differences between the (*a*) type structures and the normal ones are nearly equal at 43·5, 42·6, and 42·6 kcals./mole for azobenzene, benzylideneanil, and stilbene; and the differences for the (*b*) type structures are likewise equal; so the resonance energies expected would be nearly equal in all these compounds; (ii) the extra resonance energy calculated in benzylideneanil from the observed Q_c rises to 15·2 (uncorr.) or 9·6 (corr.) kcals./mole, and so is nearly equal to those in azobenzene and in stilbene; (iii) the resonance energy in dibenzylideneazine becomes 24·4; (iv) the extra resonance energy in dibenzylidene-ethylenediamine is calculated as 7 (uncorr.) to 2·6 (corr.) kcals./mole; (v) that in pyridine is calculated to be 37·3 kcals./mole.

The use of this lower value, therefore, resolves several anomalies; but it would mean: (i) that while our values for the heats of combustion of azo*iso*propane, benzylideneanil, dibenzylideneazine, and dibenzylidene-ethylenediamine are probably good, that of *n*-butyl*iso*-butylideneamine is 10 kcals./mole (0·8%) too low, *i.e.*, by about three times the random error which we deem probable; or (ii) that certain bond energies which we have supposed to be constant are not so; or (iii) that some of the bond energy terms used are slightly in error.

It has been shown that the anomalies exist whether or not the resonance energies are corrected for known constitutional effects. Furthermore, uncertainties in certain secondary terms such as that in the C–N bond are irrelevant to the present discussion. The resonance energy of azobenzene, for example, may be expressed: R(azobenzene) = H_a(azobenzene, obs.) − H_a(azo*iso*propane, obs.) − $6H_a$(C=C) − $2H_a$(C–C) + $4H_a$(C–H). Similar expressions may be written for the resonance energies of benzylideneanil, dibenzylidene-ethylenediamine, and pyridine.

We conclude, therefore, that the anomalies are due either to causes (i) or (ii) of those

* Structures (*d*) or (*d'*) prove less likely to be important, because they would require the molecule to



be axially symmetrical, which examination of the crystal structure has shown it not to be in stilbene and azobenzene.

considered. The possibility of the experimental value for *n*-butyl*iso*butylideneamine being erroneous can only be eliminated by further measurements on this and similar compounds.*

It is quite possible that the energy terms for the C=N and the C–N bond vary from compound to compound.† The aliphatic amines give a range of values for the latter (see above). Furthermore, the C=O bond term differs in formaldehyde, acetaldehyde, and the ketones by 13 kcals./mole (Pauling, *op. cit.*, and above).

We incline, therefore, to believe that this last explanation is the most probable; and we ascribe a value of 96—106 kcals./mole to the apparent heat of forming the C=N bond.

TABLE II.

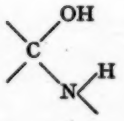
Bond energy terms.

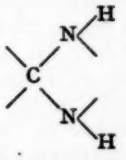
	(A)	(B)		(A)	(B)		(A)	(B)
C—C	60·3	83·0	N—N	20·4	39	C—N	52·5	73·1
C=C (1-ene)	101·2	146·5	N=N	63·1	100·3	C=N	96—106	137·5—147·5
C=C (*cis*-3-ene)	102·9	148·2				C≡N (HCN)	145·2	207·2
C≡C (1-yne)	132·7	200·5	N≡N	170	226	C≡N (cyanides)	151·6	213·6
C≡C (2-yne)	136·3	204·1						
	(A)	(B)					(A)	(B)
C—O	74·1	85·5	O—O	34·9		C—H	87·15	98·45
C=O (formaldehyde)	143	166·3	O=O	(96)		N—H	83·7	93·0
C=O (acetaldehyde)	153·4	176·7	O—H	110				
C=O (ketones)	156	179·3						

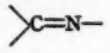
(A) based on low values for atomisation of carbon and nitrogen.
(B) on high values (p. 1189).

Some Applications of the Results.—Addition and condensation reactions. These bond energy terms are of interest in connection with certain organic reactions for they enable us to calculate roughly the heats of reaction ($-\Delta H$) in the vapour phase for alternative courses, and thus to predict the probable course. No exact correlation can be expected because (*a*) the range of the calculated ΔH values is rather large (see below), (*b*) the reaction heats in condensed phases will be somewhat different, (*c*) changes of entropy may have an important effect, (*d*) dehydrating agents, which are often used, can increase the heat evolved by about 10 kcals. per mole of water formed. It is nevertheless of interest to see to what extent such simple predictions are useful.

The common reactions of primary amines with carbonyl compounds produce one of the groupings (1)—(3); (3) may follow from (1) (cf. Sprung, *Chem. Reviews*, 1940, **26**, 297).

(1) 

(2) 

(3) 

For (1), as the value of H_a for >C=O varies from 143 to 156, $-\Delta H$ ranges from 9·9 to − 3·1 kcals./mole; for (2) correspondingly (if H_a for C–N be taken as 52·5) from 14·6 to 1·6 kcals./mole; while for (3), if H_a for C=N be 96, it ranges from + 5·6 to − 7·4 kcals./mole, or, if H_a(C=N) be 106, from 15·6 to 2·6 kcals./mole.

We should, therefore, expect that the grouping (1) would be produced only by aldehydes, and then possibly only as an unstable intermediate, while the imino-group would be formed only if its individual heat of formation is near the upper limit (106 kcals./mole), or if it is stabilised by resonance, as in hydrazones, oximes, anils, or hydrobenzamide. As we have seen, the thermal data suggest that the >C=N– bond heat of formation will be large in imines only when it has large alkyl groups attached.

The grouping (2) is otherwise the most likely to be formed. Still more energy per N atom obviously can be liberated by further condensation to give a ring or chain product, with the

* Since the foregoing was written, Dr. H. D. Springall, using the bomb calorimeter at Manchester, has very kindly measured the heat of combustion at constant pressure of a sample of liquid *iso*butyl-*n*-butylideneamine supplied by Mr. K. B. Everard and one of the present authors (L. E. S.). He found a value of 1295 ± 2·5 kcals./mole which agrees within the limits of experimental error with the value which we found for the isomer (1295 ± 2·4).

† It may be noted that we are concerned here only with the variation of their *sum*.

‡ These would favour the formation of OH groups, for the hydrogen-bonding which results therefrom would stabilise the system.

repeating grouping $-\overset{|}{\underset{|}{N}}-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{N}}-\overset{|}{\underset{|}{C}}-$. If the condensation is with ammonia, three C–N bonds per N atom might be formed. This, however, would produce a highly ordered structure, so it is likely to occur only if the molecules are small, *i.e.*, if the numbers of configurations which they can have relative to each other is small enough to make the attainment of the desired one reasonably probable. This may be one reason why it happens only with formaldehyde, a further reason being that the internal energy change is then greatest. The experimental facts are, on the whole, in agreement with these conclusions.

A reaction which appears to be made possible by resonance stabilisation in the product is the isomerisation of an azo-compound to a hydrazone. This involves the change $-N{=}N-CH_2- \longrightarrow -NH-N{=}CH-$ for which $-\Delta H$ is calulated to be only $-5{\cdot}8$ to $+4{\cdot}2$, if resonance energy be ignored. It may be remarked, however, that the azo-compounds for which this change is best known (see Taylor and Baker, " Sidgwick's Organic Chemistry of Nitrogen ", Oxford Univ. Press, 1937, p. 434) are those with one group aromatic, *e.g.*, $Ph{\cdot}N{:}N-CH_2-CH_3$, wherein there would be resonance stabilisation in both reactant and product, but more in the latter.

Polymerisation: resonance energy in polyene systems. Sidgwick (*loc. cit.*) drew attention to the fact that for the single, double, and triple bonds in the carbon–carbon series the ratios of the heats of formation are as $1 : <2 : <3$, whereas for the nitrogen–nitrogen series they are as $1 : (>2) : >3$, while for the carbon–nitrogen series they appear to be fairly accurately as $1 : (2) : 3$. The exact values of the ratios depend upon the latent heat values taken for carbon and nitrogen. The qualitative nature of the relation is independent of these, however; and so, too, are the calculated heats of the bond reactions (ignoring resonance energies) (*a*) $X{=}Y \longrightarrow 2X-Y$ or (*b*) $3X{\equiv}Y \longrightarrow 3X{=}Y + 3X-Y$, which are given in Table III. The tendencies to

TABLE III.

$-\Delta H$ (kcals./mole).

	C—C bonds.	N—N bonds.	C—N bonds.	C—O bonds.
(*a*)	19·4	− 22·3	−1 to +9	−7·8 to +5·2
(*b*)	87·3	−259·5	−9·3 to +20·7	—

polymerise, or not, are clearly marked save in the carbon–nitrogen and carbon–oxygen series. Polymerisation in these two will turn upon the bond energy term value for the particular compound considered, on temperature and concentration, and on possible resonance or steric energy, or the complication in shape of the molecules.

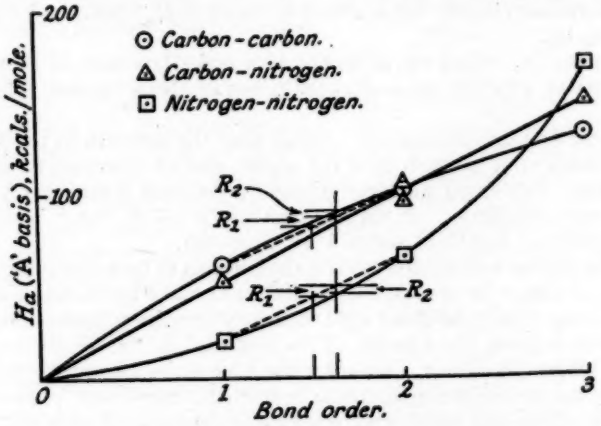
If, after the fashion of Fox and Martin (*J.*, 1938, 2106), the curves of bond energy against bond order are drawn for carbon–carbon, carbon–nitrogen, and nitrogen–nitrogen bonds, the accompanying figure is obtained; and it might be expected, following these authors' line of thought, that from bond orders as defined by Penney (*Proc. Roy. Soc.*, 1937, *A*, **158**, 306) the heats of formation of bonds in polyene systems could be derived by interpolation.* Were this so, the resonance energy in, *e.g.*, benzene would be six times the difference between the ordinate from the carbon–carbon curve, for order 1·623, and the ordinate for order 1·5 from the *chord* drawn between the points on this curve for orders 1 and 2. On this view, we could say that it arises partly from the change in order, which changes the ordinate obtained from the chord (and so gives rise to the contribution R_1), and partly from the effect of curvature, which gives rise to the additional contribution R_2. Now Penney defines the bond order as a function of the spin vectors of the π electrons on the six atoms on the ring: it is, therefore, independent of the nature of the atoms. The same is true of the bond order as defined by Coulson (*Proc. Roy. Soc.*, 1939, *A*, **169**, 413) provided that the atoms are all the same. For the hypothetical molecule N_6 the bond order would also be 1·623, but because the curvature of the energy–order graph for nitrogen–nitrogen bonds is positive, R_1 and R_2 are opposed; so there should be much less resonance energy—roughly one-quarter of that in benzene.

Further consideration of the problem shows, however, that no such simple interpretation of energy–order curves is theoretically justified (Cottrell and Sutton, *Quart. Reviews*, in the press). Penney intended his bond order to be applied to the chord between the points for order 1 and 2; and Coulson applied his order either to the chord or to a curve giving a correction for compression of the single bond and for stretching of the double one, which curve would have *positive* curvature

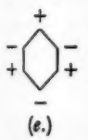
* This could only be done absolutely if the correct latent heat of vaporisation of graphite to quadrivalent carbon were known; for the slope and curvature of the energy–order curve depends on it.

instead of the negative curvature apparent in the figure for carbon–carbon bonds. Furthermore, it is not even an empirical truth that the correct bond energy in benzene can be obtained by interpolation in the way first indicated: the value so obtained is too high. If Pauling's bond order (1·5) be used, the value is too low. For the present, therefore, the only conclusion to be drawn is that measurements of resonance energy in azines and azoles are badly needed to throw light on this question.

In passing, it may be noted that the definition of bond order is less simple for carbon–nitrogen bonds than for the others. The electronegativities of carbon and nitrogen being different, there may be polar contributions in these bonds. Penney's definition of bond-order is based on the electron-pair-bond approximation and it therefore takes no account of polar structures. The molecular orbital method brings these in, though arbitrarily; so it might be expected that Coulson's definition (*loc. cit.*), based on this approximation, would be more suitable.



The part of this bond order due to π electrons, the mobile bond order, is, however, a function of the products of the coefficients a_r, a_s, etc., of the atomic orbitals required to build up the molecular orbital; it is large only when the products of the coefficients for neighbouring atoms are simultaneously large: the products for non-neighbours do not count. For the extreme case of a fully ionic structure (*e*) the mobile bond order would, therefore, be zero; and it is a maximum when a_r, a_s, etc., are all equal, *i.e.* it is really a measure of the covalent bond order, and in fact approximates very closely to the order as defined by Penney (in benzene it is 1·667). The use of such energy–order curves as the above to discuss resonance energy is even less justified, therefore, when the system contains carbon–nitrogen bonds.


(*e.*)

Bond energies. In conclusion, some remarks will be made about the absolute values of the bond energy terms, *i.e.*, regarded as bond energies. Values on both the A and the B basis will be considered in the hope of reducing the uncertainty of the discussion.

For this purpose, the B values are probably more useful, because for C–H and C–C they are roughly equal to the observed bond energies 103 and 83 respectively (Butler and Polanyi, *Nature*, 1940, **146**, 129; Bangham, *ibid.*, 1941, **147**, 542; Stevenson, *loc. cit.*; Anderson and Kistiakowsky, *loc. cit.*). In fact, the effective quadrivalent atomisation energy for carbon may be taken to be 170—190 kcals./g.-atom from these observations. A similar "practical" basis for nitrogen is still lacking.

On either basis, the absolute differences between the heats of forming double and single bonds are not very different for carbon–carbon, nitrogen–nitrogen, carbon–nitrogen (lower limit for C=N), and possibly oxygen–oxygen (see Table IV). That for carbon–oxygen is, however, markedly different; and that for carbon–nitrogen is also appreciably different if the upper limit value for C=N be taken. The differences between the values for triple and double bonds vary greatly.

The difference, Δ, between H_a(C=N) and the arithmetic mean of H_a(C=C) and H_a(N=N) is almost the same (12·5 kcals./mole) as the Δ value for single bonds (12·1), if the lower-limit value of 96 for H_a(C=N) be used. This would mean that for the second bond in the C=N bond the effective electronegativity difference between carbon and nitrogen is zero. If the upper limit of 106 be used, the double-bond difference is about twice as great (22·5) as the single-bond one, so

TABLE IV.

	H_a (X=X)—H_a (X—X).		H_a (X≡X)—H_a (X=X).	
	(A)	(B)	(A)	(B)
C—C	40·9	63·55	31·2	53·9
N—N	42·7	61·3	104·3	123·1
C—N	43·5—53·5	64—74	45—55·5	66—76·5
O—O	(61)			
C—O	69—82	81—94		

the electronegativity difference for the second bond of the double bond is then the same as that for the first (0·7).

The H_a values for C—O and C=O exceed the corresponding arithmetic means by 26·5 and 51, *i.e.*, the effective electronegativities are much the same for both parts of the double bond (0·9); H_a(C≡N) is almost exactly the arithmetic mean of H_a(C≡C) and H_a(N≡N) [151·2 (A) or 213·2 (B) kcals./mole].

The variation in the H_a values for triple bonds is much less than in those for single bonds; H_a(N≡N) is 1·28 (A) or 1·13 (B) times H_a(C≡C), but H_a(N—N) is only 0·34 (A) or 0·47 (B) of H_a(C—C).

These data suggest certain conclusions. One is that the strength of the $p\pi$ bond in C=C and N=N is nearly independent of the nature of the atoms, and of the strength of the σ bond. This may be so in C=N also; but under some conditions the $p\pi$ bond is stronger, presumably because it develops partial ionic character. It may be true also in O=O; but it certainly is not in C=O, again probably because the bond has partial ionic character.

In going from the double to the triple bonds, there seems to be a drastic reorganisation. This is not altogether surprising. In the double-bonded state, the σ bond can be formed by a variety of atomic orbitals, from p to hybridised sp^2: the only condition is that one p orbital on each atom shall be left free to form the π bond. This may explain the relatively constant character of the first π bond. When, however, a second π bond has to be formed, to give a triple bond, the bonding orbitals for the σ bond are much more restricted: they must be formed from one s and one p atomic orbital. This may involve a considerable change in the σ bond.

The similarity and regularity of the three triple-bond energy terms may arise from this fixation of atomic bonding orbitals; and they may also indicate that the C≡N bond is predominantly covalent. Nitrogen is more electronegative than carbon; so in a tertiary amine there is some piling up of negative charge on the nitrogen atom. In a triple C≡N bond, there is, however, a further compression of the bonding electrons, and the repulsion energy which this causes might reduce the contribution per electron to ionic character. The polarity of the C≡N bond may arise largely from the unshared nitrogen electrons.*

The surprisingly large ratio of H_a(N≡N) to H_a(N—N), *viz.*, 8·33 (A) or 5·8 (B), has been said to show that the N≡N bond is abnormally strong. As we have seen, however, it is not much stronger than C≡C; so a more significant observation appears to be that the N—N is abnormally weak compared with C—C. The reason for this weakness is not at present clear. Obviously it is insufficient to say that the atomic bonding orbitals used by nitrogen may be p orbitals, which form less strong bonds than do the sp^3 orbitals which must be used by tetrahedral carbon, unless we explain why these poor orbitals are used. The usual point made is that nitrogen forms only three bonds, so hybridisation is less complete; but the weakness is so pronounced that this explanation hardly seems adequate. The same problem appears to arise in the O—O bond.

The authors wish to thank Professor C. A. Coulson for helpful discussions, Messrs. T. L. Cottrell and L. Orgel for checking some of the calculations, and Imperial Chemical Industries Ltd. for a grant toward the cost of the research.

THE DYSON PERRINS AND THE PHYSICAL CHEMISTRY LABORATORIES, OXFORD.
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* Pauling (*loc. cit.*) offers the alternative explanation that the N≡N bond is largely ionic.

236. *The Application of the Method of Electrostatic Energy Differences. Part I. Stereochemistry of the Diterpenoid Resin Acids.*

By D. H. R. Barton and (in part) G. A. Schmeidler.

The potential applicability of dissociation-constant measurements in the elucidation of molecular structure is emphasised. As an example of the utility of this method of approach it is shown that rings A and C of abietic acid must be *trans*-fused, previous conflicting evidence on this point being without value.

The use of dissociation-constant measurements in the elucidation of the configuration of symmetrical dicarboxylic acids in solution has received much attention, particularly in the thorough investigations of Ingold (see Gane and Ingold, *J.*, 1931, 2153; Ingold, *ibid.*, p. 2179, and references there cited). The general interpretation of the experimental results may be conveniently summarised by the equation

$$\log_{10} K^2_{e'}/4K^2_{e''} = N_0\varepsilon^2/2{\cdot}3RTD_{\mathbf{r}}r \quad \ldots\ldots\ldots \text{(i)}$$

where $K^2_{e'}$ and $K^2_{e''}$ are respectively the first and the second measured thermodynamic dissociation constants, N_0 is the Avogadro number, ε the electronic charge, R the gas constant, and $D_{\mathbf{r}}$ the effective dielectric constant for the space between the two carboxyl groups separated by the distance r. The derivation of this equation from simple electrostatic and statistical theories is well known (Bjerrum, *Z. physikal. Chem.*, 1923, **106**, 219; Maxwell and Partington, *Trans. Faraday Soc.*, 1937, **33**, 670) and is readily extended to the relative dissociation constants of an unsubstituted acid as compared with the same acid substituted by various unit polar and dipolar groups. In the latter case we may write

$$\log_{10}\frac{K^0_{e'}}{K^s_{e'}} = \frac{N_0\varepsilon}{2{\cdot}3RT}\left[\sum_{i=1}^{i=n}\frac{\varepsilon}{D_{\mathbf{r}_i}r_i} + \sum_{j=1}^{j=m}\frac{\mu_j\cos\theta_j}{D_{\mathbf{r}_j}r_j^2}\right] \quad \ldots\ldots \text{(ii)}$$

where $K^0_{e'}$ is the measured thermodynamic dissociation constant of the unsubstituted acid, $K^s_{e'}$ refers in the same way to the acid when substituted by n unit polar groups and m dipolar groups, μ_j is the dipole moment of the jth substituent, and θ_j is the angle that the line joining the seat of the charge in the acidic group to the centre of the dipole makes with the dipole itself. The other symbols have the obvious significance and the appropriate attention must be paid to the signs to be attached to each term.

It is convenient when dealing with unit polar substituents to define

$$N_0\varepsilon^2/2{\cdot}3RTD_{\mathbf{r}}r = p\omega = -\log_{10}\omega \quad \ldots\ldots\ldots \text{(iii)}$$

There has been a number of interesting attempts to calculate values of $p\omega$ in suitable cases from first principles. Of these, the most comprehensive is that of Kirkwood and Westheimer (*J. Chem. Physics*, 1938, **6**, 506, 513; compare Sarmousakis, *ibid.*, 1944, **12**, 277), which expresses $D_{\mathbf{r}}$ as a function of the molecular dimensions and of the external and internal dielectric constants. Although this theory has, on the whole, been well received (compare, however, Wynne-Jones and Rushbrooke, *Trans. Faraday Soc.*, 1944, **40**, 99, and see also Westheimer and Kirkwood, *ibid.*, 1947, **43**, 77), it is still too complex and too unreliable to be applied by the organic chemist in the elucidation of molecular structure. Nevertheless, this does not prevent the application of tables of $p\omega$ values provided these are drawn from the empirically determined dissociation constants of suitable model substances. Such use may be described as "The Application of the Method of Electrostatic Energy Differences". In Table I various $p\omega$ values are recorded for subsequent employment in this paper.

Table I.*

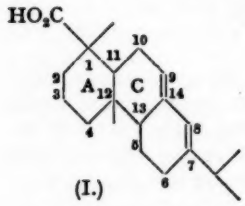
Substance.	Solvent.	$p\omega$. *cis.*	$p\omega$. *trans.*
*cyclo*Hexane-1 : 2-dicarboxylic acid	H_2O	1·80	1·15
	50 : 50-MeOH–H_2O †	2·34	1·33
1 : 2 : 3 : 4-Tetrahydronaphthalene-2 : 3-dicarboxylic acid	H_2O	1·89	1·10
*cyclo*Hexane-1 : 3-dicarboxylic acid	H_2O	0·76	0·82
	50 : 50-MeOH–H_2O †	0·98	1·32

* The $p\omega$ values given are calculated from the results of Speakman (*J.*, 1941, 490) and of Kuhn and Wassermann (*Helv. Chim. Acta*, 1928, **14**, 50). When the carboxyls are in the 1 : 2-positions this may be indicated by writing $p\omega_{1:2}$, and similarly for $p\omega_{1:3}$.

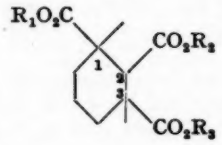
† By volume.

The first application of this method has been made in the diterpenoid resin acid field. The formula (I) for abietic acid has received abundant confirmation in recent years, but the nature of the ring fusion between rings A and C is still uncertain as also is the stereochemistry at C_{13}. With regard to the former problem, Campbell and Todd (*J. Amer. Chem. Soc.*, 1942, **64**, 928) have suggested that the fusion is *trans*-, whilst Lombard (*Bull. Soc. chim.*, 1946, **13**, 428) asserts that it is *cis*-. Campbell and Todd's evidence is based upon a study of the relative degree of steric hindrance towards alkaline hydrolysis of the esters of podocarpic acid and dehydroabietic acid. It is only correct if *cis*-decalin is assumed to have the conventional Sachse–Mohr configuration and not that of the energetically more stable two-chair *cis*-decalin (see Barton, this vol., p. 340, where these two *cis*-decalins are conveniently illustrated). This may easily be seen by an inspection of molecular models. Since the latter alternative is the more probable, Campbell and Todd's reasoning is not admissible.

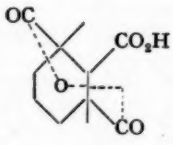
Lombard's conclusion is based on the following evidence. When abietic acid is vigorously oxidised it furnishes, amongst other products, a tricarboxylic acid, $C_{11}H_{16}O_6$, to which the formula (II; $R_1 = R_2 = R_3 = H$) must be assigned. This acid has been studied in some detail by Ruzicka, Goldberg, Huyser, and Seidel (*Helv. Chim. Acta*, 1931, **14**, 545), who showed that the 1 and the 3 carboxyl group must be in the *cis*-relationship. On treatment with acetyl chloride the tricarboxylic acid furnished a mixture of two anhydrides, m. p. *ca.* 100° and m. p. 170—172°. On symmetry grounds the higher-melting anhydride must now be given the formula (III) and the lower-melting one the formula (IV). [Formulæ (II), (III), and (IV) have no stereochemical significance as written here.] Although both these anhydrides could be smoothly reconverted into the parent tricarboxylic acid by hydrolysis, the method of their formation with acetyl chloride does *not* provide evidence as to the stereochemistry of the carboxyl attached to C_3 (compare Werner and Conrad, *Ber.*, 1899, **32**, 3046).



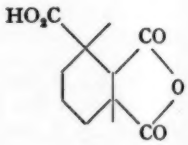
(I.)



(II.)



(III.)



(IV.)

Subsequently, Lombard (Thesis, Paris, 1943, p. 43) showed that the action of heat alone at about 250° on the tricarboxylic acid furnished an anhydride, m. p. 175°, admitted to be identical with that of m. p. 170—172° prepared earlier by Ruzicka *et al.* (*loc. cit.*). If this anhydride had been derived from two carboxyls attached at the 1 : 2-positions in the *cyclo*hexane ring its method of formation would indeed have proved them to be in the *cis*-relationship. Since, however, it is doubtless derived from the 1 : 3-carboxyls, its formation by the action of heat only serves to confirm that these are in the *cis*-relationship (it is well known that *trans-cyclo*hexane-1 : 3-dicarboxylic acid does not furnish a monomeric anhydride) and provides no evidence as to the stereochemistry of the carboxyl attached at C_3.

In the discussion of polybasic acids in this paper and subsequent parts of this series it is convenient to define nomenclature for dealing with the general case. For a completely asymmetric n-basic acid, AH_n, there are n possible sites of residence for the negative charge on derived univalent anions and $n(n-1)/2$ sites of residence for the two negative charges on derived bivalent anions. The first two measured (or gross) thermodynamic dissociation constants, $K^n_{a'}$ and $K^n_{a''}$, may be defined by

$$K^n_{a'} = a_{H^+} \cdot \frac{\sum_{j=1}^{j=n} [AH_{n-1}]^-_j \cdot f_-}{[AH_n] \cdot f_0} = \sum_{k=1}^{k=n} {}^kK^n_{a'} \quad \text{(iv)}$$

and

$$K^n_{a''} = a_{H^+} \cdot \frac{\sum_{i=1}^{i=n(n-1)/2} [AH_{n-2}]^=_i \cdot f_=}{\sum_{j=1}^{j=n} [AH_{n-1}]^-_j \cdot f_-} = \sum_{i=1}^{i=n(n-1)/2} \frac{1}{\sum_{m=1}^{m=n} \frac{1}{{}^iK^n_{a''}}} \quad \text{(v)}^*$$

* The use of the expression on the extreme right in (v) will be found to involve partial dissociation constants that do not *formally* exist. This difficulty is, however, easily resolved since the latter can be expressed in terms of those partial dissociation constants which do have obvious meaning.

The expression on the extreme right in (iv) represents the sum of the thermodynamic *partial* dissociation constants for the first ionisation, whilst the corresponding expression in (v) represents $K^n_{a''}$ in terms of the thermodynamic partial dissociation constants for the second ionisation. The generalised nomenclature adopted for partial dissociation constants will be clear from their context.* The symbols f_0, f_-, and $f_=$ represent the activity coefficients of the undissociated acid, the univalent anions, and the bivalent anions, respectively.

If one now considers the potentiometric titration of this n-basic acid, assuming that higher charges than two do not make contributions during the addition of B equivs. of alkali (up to a total of 2 equivs.), then

$$A = \text{molarity of the acid} = [AH_n] + \sum_{j=1}^{j=n} . [AH_{n-1}]^-_j + \sum_{i=1}^{i=n(n-1)/2} [AH_{n-2}]^=_i$$

and

$$B + [H]^+ = \sum_{j=1}^{j=n} [AH_{n-1}]^-_j + 2 \sum_{i=1}^{i=n(n-1)/2} [AH_{n-2}]^=_i + [OH]^-$$

Defining

$$\alpha = B + [H]^+ - [OH]^- = \sum_{j=1}^{j=n} [AH_{n-1}]^-_j + 2 \sum_{i=1}^{i=n(n-1)/2} [AH_{n-2}]^=_i$$

$$\beta = A - B - [H]^+ + [OH]^- = [AH_n] - \sum_{i=1}^{i=n(n-1)/2} [AH_{n-2}]^=_i$$

$$\gamma = 2A - B - [H]^+ + [OH]^- = 2[AH_n] + \sum_{j=1}^{j=n} [AH_{n-1}]^-_j$$

it is readily shown that

$$a^2_{H^+} . \left(\frac{\alpha}{\gamma}\right) . \frac{f_=}{f_0} = K^n_{a'} . a_{H^+} . \left(\frac{\beta}{\gamma}\right) . \frac{f_=}{f_-} + K^n_{a'} . K^n_{a''} \quad . \quad . \quad . \quad . \quad . \quad . \quad \text{(vi)}$$

Clearly this may be plotted as a linear equation and has, therefore, considerable advantages in the interpretation of the experimental results. All the data recorded in the experimental section for polybasic acids were obtained in this way. For a symmetrical dibasic acid (vi) takes a form previously shown to hold by Speakman (*J.*, 1940, 855), whilst for asymmetrical dibasic acids (vi) assumes a form, (vii), previously shown to be applicable by the author (*Nature*, 1947, **160**, 752):

$$a^2_{H^+} . \left(\frac{\alpha}{\gamma}\right) . \frac{f_=}{f^-} = ({}^1K^2_{a'} + {}^2K^2_{a'}) . a_{H^+} . \left(\frac{\beta}{\gamma}\right) . \frac{f_=}{f_-} + \frac{({}^1K^2_{a'} + {}^2K^2_{a'}) . {}^1_1K^2_{a''} . {}^1_2K^2_{a''}}{({}^1_1K^2_{a''} + {}^1_2K^2_{a''})} \quad . \quad . \quad . \quad \text{(vii)}$$

(vi) may be used for the evaluation of pω values and hence for the elucidation of molecular structure. In the important case of an asymmetric dicarboxylic acid [equation (vii)] the constant term may be simplified by the considerations that

$$\log_{10} \frac{{}^1K^2_{a'}}{{}^1_2K^2_{a''}} = \frac{N_0\varepsilon^2}{2{\cdot}3RTD_{\mathbf{R}\gamma}} = \log_{10} \frac{{}^2K^2_{a'}}{{}^1_1K^2_{a''}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \text{(viii)}$$

so that it becomes equal to

$${}^1K^2_{a'} . {}^2K^2_{a'} . e^{-N_0\varepsilon^2/RTD_{\mathbf{R}\gamma}}$$

from which pω may be calculated provided that ${}^1K^2_{a'}$ or ${}^2K^2_{a'}$ is known. Measurements on (say) monomethyl esters readily provide this information.

Before proceeding further with application of (vi) it is of interest to see how far $K^n_{a'}$ values are in agreement with those calculated from the dissociation constants of the appropriate methyl esters in the case of the tricarboxylic acid (II; $R_1 = R_2 = R_3 = H$) and its derivatives. It will be seen from Table II that agreement is satisfactory, the carboxyl group having an apparently more powerful " inductive effect " than the carbomethoxyl group, an observation in full accord with the data for comparable acids (see Greenspan, *Chem. Reviews*, 1933, **12**, 339).

* Thus the left-hand superscript refers to the ions, numbered in order, produced by the dissociation, the left-hand subscript to the dissociating molecules or ions numbered in order, the right-hand superscript to the number of dissociatable carboxyl groups in the initial polybasic acid, and the right-hand subscript to the stage of dissociation—first stage, one dash; second stage, two dashes, and so on. The subscript a indicates that reference is made to thermodynamic partial dissociation constants. For the first dissociation the left-hand subscript may be omitted, since it is unity for all n values of the left-hand superscript. Similarly for the last dissociation the left-hand superscript may be omitted without ambiguity.

TABLE II.*

Substance.	$K^n_{a^{n\cdot}} \times 10^5$. Obs.	Calc. from dimethyl esters (see Experimental).
(II; $R_1 = R_2 = R_3 = H$)	14·0	11·8
(II; $R_1 = Me$, $R_2 = R_3 = H$)	10·3	8·8
(II; $R_2 = Me$, $R_1 = R_3 = H$)	6·54	6·04

* All data are for aqueous solutions.

The asymmetric monomethyl ester (II; $R_1 = Me$, $R_2 = R_3 = H$) of the tricarboxylic acid being considered, application of equations (vii) and (viii) should enable a $p\omega_{1:2}$ value to be obtained for comparison with those given in Table I. A *cis*-dicarboxylic acid should have a $p\omega_{1:2}$ value of about 1·85, a *trans*-acid a value of about 1·13. In practice a value of $p\omega_{1:2} = 1{\cdot}11$ was found, which is proof that the C_2 carboxyl in the tricarboxylic acid has the *trans*-configuration with respect to the C_1 and C_3 carboxyls.

Now it is easily seen that the nth measured thermodynamic dissociation constant of an n-basic acid is related to the corresponding partial dissociation constants by the equation

$$\frac{1}{K^n_{a^{n\cdot}}} = \sum_{i=1}^{i=n} \frac{1}{{}_iK^n_{a^{n\cdot}}} \quad \ldots\ldots\ldots\ldots \quad \text{(ix)}$$

For the tricarboxylic acid (II; $R_1 = R_2 = R_3 = H$) it is clear, having regard to symmetry conditions, that the equation

$$\frac{1}{K^3_{a'''}} = \frac{2}{{}_1K^3_{a'''}} + \frac{1}{{}_2K^3_{a'''}} \quad \ldots\ldots\ldots\ldots \quad \text{(x)}$$

must hold. Since ${}_1K^3_{a'''}$ and ${}_2K^3_{a'''}$ can be calculated from the $K^1_{a'}$ values of the appropriate dimethyl esters (see Tables IV and V) and the appropriate $p\omega$ values (see Exptl.), calculated values of $pK^3_{a'''}$ may be compared with those observed experimentally. This information is presented in Table III. It will be seen that the observed values are in satisfactory agreement for a *cis*-structure in the tricarboxylic acid, a result in apparent conflict with the evidence given above. In actual fact there is no disagreement, for the following reasons.

TABLE III.

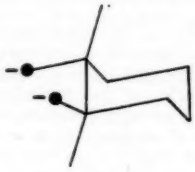
Solvent.	$pK^3_{a'''}$. *cis.*	*trans.*	Obs.	Remarks.
H_2O	8·03	7·09	7·83	Determined potentiometrically
50 : 50-MeOH–H_2O (by vol.)	10·23	8·86	~9·6	This observed value is only an approximate one for $pK^3_{a'''}$ determined colorimetrically

It is now generally accepted that the chair configuration of *cyclo*hexane is appreciably more stable than the boat configuration (compare Barton, *loc. cit.*). In the former all arrangements of the valency directions about the carbon atoms correspond to the staggered configuration in ethane, in which there are two distances between hydrogen atoms not bonded to the same carbon atom, a " short " distance and a " long " distance. In the chair configuration of *cis-cyclo*hexane-1 : 2-dicarboxylic acid the negative charges of the bivalent anion must be separated by the " short " distance [as in (V)] whilst in the *trans*-acid the distance may be either " short " or " long " [as in (VI*a*) and (VI*b*) respectively]. The fact that *cis*- and *trans*-acids have greatly different $p\omega_{1:2}$ values is, on the assumption that D_x is roughly the same for both, a proof that the configuration of the bivalent anion of the *trans*-acid must be as in (VI*b*) (compare Speakman, *J.*, 1941, 490), which is, of course, energetically more stable on electrostatic reasoning than (VI*a*). Now the $p\omega_{1:3}$ value for *cis-cyclo*hexane-1 : 3-dicarboxylic acid in water from Table I is 0·76, whilst that for the symmetrical monomethyl ester (II; $R_2 = Me$, $R_1 = R_3 = H$) (see Exptl.) is in fair agreement at 0·96. The discrepancy is doubtless due to the lower value of D_x in (II; $R_2 = Me$, $R_1 = R_3 = H$) because here more of the lines of force in the bivalent anion travel through the bulky carbomethoxyl group of low dielectric constant than through the relatively small hydrogen atom in the reference compound. The bivalent anions of such *cyclo*hexane-1 : 3-dicarboxylic acids can adopt two configurations shown in (VII*a*) and (VII*b*). In (VII*a*) the charges (assumed to lie 1 A. beyond the C atom of the COO^- group and on the line of the terminal C–C bond : compare Gane and Ingold, *loc. cit.*) are 2·51 A. apart, whilst in (VII*b*) they are 6·66 A. apart, so that (VII*b*) is clearly the configuration to be expected. The relative

$p\omega_{1:2}$ and $p\omega_{1:3}$ values confirm this. The calculations of Table III have been made assuming that the *cis*-tervalent anion would have the configuration (VIII*a*) and the *trans*-anion that in (VIII*b*), both configurations being logical extensions of (V) and (VI*b*) respectively.

It is, however, possible for the *trans*-anion to adopt the configuration (VIII*c*), in which the distance between the negative charges 1 and 2 and 2 and 3 (4·02 A.) corresponds to the " short " distance and not the " long " distance (5·78 A.) as in (VIII*b*). In this case the $pK^3_{a_{\infty}}$ value for the tricarboxylic acid would approximate to that required by the *cis*-anion (VIII*a*), as is experimentally observed. Using the $p\omega$ values of Table I for the evaluation of electrostatic energy differences

(V.)



(VI*a*.)

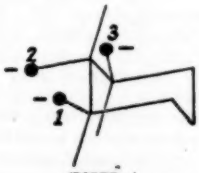
(VI*b*.)

(VII*a*.)

(VII*b*.)

(VIII*a*.)

(VIII*b*.)



(VIII*c*.)

and making the necessary extrapolation to obtain D_g for $r = 2{\cdot}51$ A., we can readily show that (VIII*c*) is about 1 kilocal. more stable than (VIII*b*). It is possible to reconcile therefore the data of Table III with the *trans*-configuration of the tricarboxylic acid (II; $R_1 = R_2 = R_3 = H$) without undue difficulty.

Finally, one can evaluate $p\omega_{1:2}$ for the tricarboxylic acid by the application of equation (vi) to the first two-thirds of its titration curve. As before, a $p\omega_{1:2}$ value of about 1·85 would indicate a *cis*-acid and a value of about 1·13 a *trans*-acid. Experimentally a value of about 1·37 was observed. This may be taken as being in fair agreement with the requirement for a *trans*-acid because the constant term in (vi), from which $p\omega_{1:2}$ is evaluated, is sensitive to errors in the determination of the second measured dissociation constant of (II; $R_2 = Me$, $R_1 = R_3 = H$).

From these experiments it is to be concluded that the ring fusion between rings A and C in abietic acid is *trans*-. The same conclusion must apply to levopimaric acid, *iso*abietic acid, neoabietic acid, and dextrosapinic acid, and also to dextropimaric acid and *iso*-dextropimaric acid. (The inter-relationship of these acids is fully dealt with in " The Terpenes ", Vol. III, by Simonsen and Barton, Cambridge Univ. Press.)

Experimental.

All m. p.s of acids which decomposed at the temperature of fusion were taken in the manner described by Ruzicka *et al.* (*Helv. Chim. Acta*, 1931, **14**, 545). Under these conditions reproducible results were obtainable.

The expression " light petroleum " refers to the fraction of b. p. 40—60°.

Tricarboxylic Acid (II; $R_1 = R_2 = R_3 = H$).—Abietic acid was prepared from colophony by Dupont's method (*Bull. Soc. chim.*, 1924, **35**, 394), which was found to be superior to that of Steele (*J. Amer. Chem. Soc.*, 1922, **44**, 1333). The abietic acid was oxidised by nitric acid according to the second procedure described by Levy (*Ber.*, 1929, **62**, 2497) except that addition, as catalyst, of 0·2% by weight of the abietic acid of vanadium pentoxide was found to be beneficial (compare Lombard, *Bull. Soc. chim.*, 1942, **9**, 833; Thesis, Paris, 1943, p. 40). A preliminary oxidation of the abietic acid by potassium permanganate was not required. The tricarboxylic acid was isolated by evaporation of the nitric acid solution at 50—60° and then readily crystallised out in a nearly pure form. It was purified by recrystallisation from acetone; m. p. 218° (decomp.) (Found: equiv., 81·0. Calc. for $C_{11}H_{16}O_6$: equiv., 81·3); yield about 7%.

Trimethyl Ester (II; $R_1 = R_2 = R_3 = Me$).—The tricarboxylic acid, suspended in ether, was esterified with diazomethane in the usual way. After removal of the ether in a vacuum and recrystallisation from aqueous methanol, an almost quantitative yield of the trimethyl ester, m.p. 73·5—74·5°, was obtained.

s-*Dimethyl Ester* (II; $R_1 = R_3 = Me$, $R_2 = H$).—Prepared by esterification of the tricarboxylic acid with methanolic hydrogen chloride according to directions given by Ruzicka *et al.* (*loc. cit.*), and recrystallised from ether–light petroleum, this had m. p. 120—120·5° (Found: equiv., 272. Calc. for $C_{12}H_{20}O_6$: equiv., 272).

as-*Dimethyl Ester* (II; $R_1 = R_2 = Me$, $R_3 = H$).—1·49 G. of the trimethyl ester (II; $R_1 = R_2 = R_3 = Me$) in methanol solution were refluxed for 18 hours with the theoretical amount of methanolic potassium hydroxide required for the hydrolysis of one carbomethoxyl group. The methanol was removed by evaporation in a vacuum, and the crystalline residue separated by addition of ether and a little water into acid and neutral fractions. The latter furnished 1·17 g. of unchanged trimethyl ester, whilst the former, after acidification, afforded a total of 210 mg. of as-*dimethyl* ester; recrystallised from benzene–light petroleum, this had m. p. 119—121°. There was a large depression in m. p. on admixture with the *s*-dimethyl ester described above (Found: C, 57·4; H, 7·6; equiv., 273. $C_{12}H_{20}O_6$ requires C, 57·4; H, 7·4%; equiv., 272).

as-*Monomethyl Ester* (II; $R_1 = Me$, $R_2 = R_3 = H$).—The tricarboxylic acid was esterified with methanolic hydrogen chloride according to Ruzicka *et al.* (*loc. cit.*), but working up of the product according to their directions afforded various preparations of m. p.s between 152° and 160°. Those of lower m. p. had equivalents that were too high, and those of higher m. p. equivalents that were too low. These products could be crystallised with difficulty from ethyl acetate–benzene on long standing. After such treatment the m. p.s were usually about 156°; Ruzicka *et al.* (*loc. cit.*) give m. p. 154—156° for a preparation described as a fine powder. It was finally found that the *as*-monomethyl ester crystallised well from water to give a product, m. p. *ca.* 163° (decomp.) (Found: equiv., 133. Calc. for $C_{11}H_{16}O_6$: equiv., 129). Repeated crystallisation from water did not change the m. p. significantly and did not greatly alter the equivalent (Found: equiv., 129, 130, 129). It is to be concluded that the preparation described here was pure *as*-monomethyl ester.

s-*Monomethyl Ester* (II; $R_2 = Me$, $R_1 = R_3 = H$).—Prepared by vigorous alkaline hydrolysis of the trimethyl ester (II; $R_1 = R_2 = R_3 = Me$) (see above) following the directions of Ruzicka *et al.* (*loc. cit.*), and recrystallised from water, this had m. p. 188—190° (decomp.) (Found: equiv., 130. Calc. for $C_{11}H_{16}O_6$: equiv., 129).

Determination of Dissociation Constants.—Thermodynamic dissociation constants in water were determined by potentiometric titration following, in general, Speakman's procedure (*J.*, 1940, 855). The measurements were made at room temperature which varied over the range 23° ± 2°. All solutions were made up in carbon dioxide-free distilled water.

A saturated calomel electrode was used as source of constant potential and was joined by means of a glass capillary containing potassium chloride solution, suitably gelled by the addition of agar-agar, to the titration cup of 10—20 ml. capacity. This cup contained a glass electrode and the drawn out tip of a mercury-operated 1-ml. micro-burette (reading to 0·001 ml.).

During titration the solution in the cup was stirred by passage of a stream of nitrogen previously saturated with water vapour. When using 0·0263N-sodium hydroxide solution delivered from the micro-burette and an acid molarity of about 0·0003 it is not necessary to correct for the change in volume during the titration. The pH scale was standardised by the buffers suggested by Hitchcock and Taylor (*J. Amer. Chem. Soc.*, 1937, **59**, 1812), and activity coefficients, *f*, were calculated from the Debye–Hückel equation $\log_{10} f = -0{\cdot}5z^2\sqrt{\mu}$, where z = valency of the ion and μ = the ionic strength. In view of the very dilute solutions employed in the experiments in this paper, the use of this equation is fully justified.

The results obtained are summarised in Table IV, the symbolism being that defined above. It is also fully explained in the text how the results of a potentiometric titration are converted into the linear equations required for the evaluation of the thermodynamic dissociation constants.

TABLE IV.

Substance.	Molarity.	K_a values.
(II; $R_1 = R_2 = R_3 = H$)	0·000460	$K^2_{a'} = 13{\cdot}3 \times 10^{-5}$; $p\omega_{1:2} = 1{\cdot}32$ *
	0·000223	$K^2_{a'} = 14{\cdot}0 \times 10^{-5}$; $p\omega_{1:2} = 1{\cdot}42$ *
		$K^2_{a''} = 1{\cdot}48 \times 10^{-8}$ † or $1{\cdot}61 \times 10^{-8}$ ‡
(II; $R_1 = R_2 = Me$, $R_3 = H$) ...	0·000293	$K^1_{a'} = 5{\cdot}75 \times 10^{-5}$
(II; $R_1 = R_3 = Me$, $R_2 = H$) ...	0·000416	$K^1_{a'} = 3{\cdot}02 \times 10^{-5}$
(II; $R_1 = Me$, $R_2 = R_3 = H$) ...	0·000570	$K^2_{a'} = 10{\cdot}3 \times 10^{-5}$; $p\omega_{1:2} = 1{\cdot}11$ *
(II; $R_2 = Me$, $R_1 = R_3 = H$) ...	0·000500	$K^2_{a'} = 6{\cdot}54 \times 10^{-5}$; $K^2_{a''} = 1{\cdot}84 \times 10^{-5}$; $p\omega_{1:3} = 0{\cdot}96$

* Values of $p\omega$ are calculated by using the result $K^1_{a'} = 3{\cdot}27 \times 10^{-5}$ obtained in the titration of (II; $R_2 = Me$, $R_1 = R_3 = H$). This is then used to obtain the other necessary figures by subtraction from $K^m_{a'}$ values.

† The Henderson equation being assumed to apply to the last third of the titration curve.

‡ On the assumption that the last two thirds of the titration curve can be analysed by the use of equation (vii) (see text).

(With C. J. W. BROOKS.) Concentration dissociation constants in 50 : 50 (by vol.) methanol–water were determined by colorimetry, approximately 0·01M-solutions, one-half neutralised with respect to the appropriate dissociation constant, being used. The match points were determined against partly neutralised solutions of benzoic acid and phenol or *p*-chlorophenol depending on the approximate pH. Reference data for these substances were taken from Bright and Briscoe (*J. Physical Chem.*, 1933, **37**, 787) and from Kuhn and Wassermann (*Helv. Chim. Acta*, 1928, **11**, 1). Table V summarises the results of the experiments.

TABLE V.

Substance.	Indicator.	Reference acid.	pK_a values.
(II; $R_1 = R_2 = R_3 = H$)	Thymol-blue	Phenol	~9·6
	Thymol-blue	*p*-Chlorophenol	~9·6
	Bromothymol-blue	*p*-Chlorophenol	~9·6
(II; $R_1 = R_3 = Me$, $R_2 = H$)	Methyl-red	Benzoic acid	~5·45
(II; $R_1 = R_2 = Me$, $R_3 = H$)	—	—	~5·73 *

* This value was assigned from consideration of that for (II; $R_1 = R_3 = Me$, $R_2 = H$). It cannot be seriously in error.

We are indebted to the Government Grants Committee of the Royal Society for financial assistance in the purchase of a pH meter. This work was carried out during the tenure of an I.C.I. Fellowship by one of us (D. H. R. B.).

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237. *Isomers of 2-Chlorovinyldichloroarsine.*

By C. L. HEWETT.

Two isomeric forms of 2-chlorovinyldichloroarsine have been prepared from the mixture obtained by the interaction of acetylene and arsenic trichloride in the presence of catalysts. The pure arsonic acids have also been prepared.

THE possibility that 2-chlorovinylarsonic acid and the derived dichloroarsine (" lewisite ") could exist in *cis*- and *trans*-forms has been mentioned in the literature (cf. Gibson and Gibson, *J.*, 1931, 753), but no attempt had been made to prepare the two forms in a pure state.

The mixture known as " lewisite ", prepared by interaction of acetylene and arsenic trichloride in the presence of aluminium chloride (Green and Price, *J.*, 1921, **119**, 448) was reported to be highly active as a vesicant, its activity being due to the 2-chlorovinyldichloroarsine. When, however, 2-chlorovinyldichloroarsine was prepared by using mercuric chloride or cuprous chloride as a catalyst (Jones *et al.*, *Ind. Eng. Chem.*, in the press), it appeared to be somewhat less vesicant and it seemed possible that this difference might be due to different amounts of the two isomers being present. Since aluminium chloride usually favours the formation of the *trans*-compound (Zelinsky and Turowa-Pollak, *Ber.*, 1929, **62**, 1618; 1932, **65**, 1299) and since acetylene normally gives rise to *trans*-addition products (Garner, *Chem. News*, 1919, **119**, 16), it seemed probable that the aluminium chloride product would have the *trans* configuration. Backe and Beute (*Rec. Trav. chim.*, 1935, **54**, 167), however, have shown that under certain conditions propiolic acid gives rise to *cis*-addition products, and it seemed possible that mercuric chloride and cuprous chloride might give rise to more *cis*-2-chlorovinyldichloroarsine than would aluminium chloride.

2-Chlorovinyldichloroarsine prepared by all three methods was shown to consist in the main of the same isomer by oxidation, by means of hydrogen peroxide, to the same arsonic acid, m. p. 130—131°, which is presumed to have the *trans*-configuration. In each case the liquors of this acid contained a second *acid* which was extremely soluble in water and in all aqueous solvents and difficult to isolate.

Attempts to separate *cis*- and *trans*-2-chlorovinyldichloroarsine by fractional distillation indicated that the isomer presumed to have the *cis*-configuration had a lower b. p. than the other, since the lower-boiling fractions yielded less arsonic acid, m. p. 130—131°, than did the higher-boiling fractions. However, since no clear cut could be observed between the first and last fractions without a slow distillation rate, this method of separation was abandoned, since prolonged heating of the dichloroarsine causes disproportionation to arsenic trichloride and the 2:2′-dichlorodivinylchloroarsine.

Pure *trans*-chlorovinyldichloroarsine, prepared from the arsonic acid, liberates two molecules of hydrochloric acid with water with formation of chlorovinylarsine oxide; on treatment with cold sodium hydroxide solution a third molecule of hydrochloric acid is liberated with the formation of sodium arsenite and evolution of one molecule of acetylene. This gives a useful method of estimating the purity of *trans*-2-chlorovinyldichloroarsine; when, however, it was applied to redistilled 2-chlorovinyldichloroarsine prepared by using cuprous chloride as catalyst, it gave results of only 90%. This suggested that the other constituent, presumably the *cis*-compound, was present to the extent of 10% and had different properties.

cis-2-Chlorovinyldichloroarsine was finally prepared by a combination of fractional distillation of the dichloroarsines, and fractional crystallisation of the arsonic acids. Crude lewisite prepared with a cuprous chloride catalyst (containing arsenic trichloride and some monochloroarsine) was oxidised by means of chlorine water and the bulk of the *trans*-compound removed as arsonic acid. The acid liquors were reconverted into the chloroarsines and fractionated; this gave a product very rich in the *cis*-isomer, which dissolved in cold sodium hydroxide solution without evolution of gas and therefore contained no *trans*-compound. Direct oxidation of the dichloroarsine gave a solution of the arsonic acid in hydrochloric acid solution from which the acid was difficult to isolate; the dichloroarsine was therefore shaken with water to give the arsine oxide, which was then oxidised by means of hydrogen peroxide solution. The solution on evaporation to dryness gave an oily solid which crystallised from acetone–carbon tetrachloride to give the pure cis-2-*chlorovinylarsonic acid*, m. p. 90—91°.

Pure cis-2-*chlorovinyldichloroarsine*, m. p. — 46°, prepared from this acid, dissolved in cold sodium hydroxide solution with formation of the sodium salt of the arsine oxide without evolution of gas, but on warming to 40° vinyl chloride was liberated, the liberation being brisk at 80°. Thus the *cis*-compound liberates no further hydrochloric acid on treatment with sodium hydroxide solution and agrees in properties with the 10% of impurity in the *trans*-compound formed by interaction of acetylene and arsenic trichloride in the presence of cuprous chloride.

An impure specimen of the *cis*-acid was isolated by similar means from the 2-chlorovinyldichloroarsine prepared by the use of mercuric chloride as catalyst.

The evidence for the configuration of this pair of isomers is given by McDowell, Moelwyn-Hughes, and Emblem (following paper); however, the *trans*-isomer normally has the higher m. p.

The *arsine oxides* of the two isomers have been prepared in a crystalline state by allowing a dilute solution of the dichloroarsine in water to stand for a long time; the *trans*-compound has m. p. 140°, and the *cis*-, m. p. 130—131°.

The *cis*-isomer can be determined in a mixture of the two by taking advantage of the fact that it dissolves in cold sodium hydroxide solution with formation of the sodium salt of the arsine oxide which can be reconverted into the dichloroarsine with hydrochloric acid, whereas the *trans*-compound decomposes to sodium arsenite. The dichloroarsine can be titrated by means of iodine in the presence of the arsenic chloride without interference. Any secondary or tertiary arsine present will not dissolve in sodium hydroxide solution and can be extracted with a solvent.

Attempts to isomerise the *trans*-dichloroarsine to the *cis*- by irradiation with ultra-violet light, which often converts the stable form into the labile, met with no success, the main reaction was disproportionation, although analysis indicated that some *cis*-compound was formed. In the presence of air the arsonic acid was formed, which was unaffected by ultra-violet light.

Treatment of the *trans*-arsonic acid with phosphorus oxychloride gave a mixture of the *cis*- and *trans*-isomers together with arsenic trichloride, *cis*- and *trans*-dichloroethylene, and tetrachloroethane.

Experimental.

trans-2-*Chlorovinylarsonic Acid*.—(*a*) 2-Chlorovinyldichoroarsine (" lewisite-I "; 182 g.) prepared by the method of Green and Price (*loc. cit.*) from arsenic trichloride, acetylene, and aluminium chloride, was oxidised by stirring it with water whilst chlorine was passed in to excess. The clear solution was evaporated under reduced pressure several times with distilled water, and the residue crystallised from water in colourless needles, m. p. 130—131°. Yield, 123 g.

(*b*) 2-Chlorovinyldichloroarsine prepared by the use of mercuric chloride as catalyst (Jones *et al.*, *Ind. Eng. Chem.*, in the press) was oxidised as above and yielded the same acid, m. p. 130—131°.

(*c*) 2-Chlorovinyldichloroarsine, prepared by using cuprous chloride as catalyst (*idem*, *ibid.*), on similar oxidation gave the same arsonic acid, m. p. 130—131°. In each case this was the preponderating product, but the liquors contained a more soluble isomer.

cis-2-*Chlorovinylarsonic Acid*.—The mixture (40 kg.) obtained by interaction of acetylene and a solution of arsenic trichloride in tetrachloroethane with cuprous chloride in triethanolamine hydrochloride solution (*idem*, *ibid.*) was distilled, and the first 16 kg. to distil collected; the boiling range was up to 60°/5 mm. The distillate was a mixture of tetrachloroethane, arsenic trichloride, *cis*-lewisite-I, *trans*-lewisite-I, and some 2 : 2′-dichlorodivinylchloroarsine (" lewisite-II "). It was stirred with its own volume of water and chlorine passed in to excess. The tetrachloroethane and arsenic trichloride were then removed under reduced pressure, and the aqueous solution concentrated; on cooling, the *trans*-arsonic acid separated and was recrystallised from water. Yield, 1·9 kg. The mother liquors were then concentrated to a syrup, dissolved in concentrated hydrochloric acid, and reduced with sulphur dioxide and a trace of iodine to the chloroarsines. The mixture was then distilled once and then fractionated twice up a 4 ft. × 1 in. column packed with glass cuts and fitted with a total reflux head operating at an 8 : 1 ratio. Separation of the *cis*-lewisite-I from the *trans*-compound and lewisite-II was sharp, and a fraction, b. p. 57·2—57·6°/7 mm., was obtained (225 g.). After removal of this fraction the temperature of the vapour rose rapidly to 65° while only 20 g. distilled; this was mainly *trans*-compound.

This crude *cis*-lewisite-I was shaken with water (1 l.), and the crude arsine oxide separated as an oil which rapidly solidified. The oxide was broken up and washed with water and added in small amounts to excess of hydrogen peroxide solution (20 vols.) cooled in ice. The clear solution of arsonic acids was then evaporated under reduced pressure to a syrup which slowly crystallised. The semi-solid mass was dissolved in acetone (40 c.c.), chloroform (20 c.c.) added, and the solution cooled to − 40°. After some hours at − 40°, the crystals were filtered off on a Buchner funnel cooled to − 40° and recrystallised from acetone at − 40°. The cis-*chlorovinylarsonic acid* separated as colourless needles, m. p. 90—91° (Found: Cl, 19·0; As, 38·2. $C_2H_4O_3ClAs$ requires Cl, 19·0; As, 40·2%. The arsenic was very difficult to determine). This arsonic acid is extremely soluble in water and difficult to crystallise when water is present.

cis-2-*Chlorovinyldichloroarsine.*—*cis*-2-Chlorovinylarsonic acid (87 g.) was dissolved in concentrated hydrochloric acid (250 c.c.) and reduced with sulphur dioxide and a trace of iodine with stirring and external cooling. The cis-2-*chlorovinyldichloroarsine* distilled as a colourless liquid (92 g.), b. p. 51—52°/5 mm., m. p. − 46·6° (Found: Cl, 51·2; As, 36·0. $C_2H_2Cl_3As$ requires Cl, 51·3; As, 36·1%). *cis*-2-Chlorovinyldichloroarsine dissolves in cold sodium hydroxide solution without evolution gas and with removal of two chlorine atoms by hydrolysis (Found: Cl, 34·35. Calc. for two Cl atoms, 34·2%); addition of hydrochloric acid regenerates the dichloroarsine. The *trans*-isomer dissolves in cold sodium hydroxide solution with evolution of acetylene and liberation of three chlorine atoms; arsenic trichloride is obtained by addition of hydrochloric acid. The solution obtained by dissolving the *cis*-isomer in sodium hydroxide solution on heating to 40° slowly liberates vinyl chloride, b. p. − 18°; no acetylene was detected.

cis-2-*Chlorovinylarsine Oxide.*—When *cis*-2-chlorovinyldichloroarsine is shaken with a large volume of water, it dissolves, and the *arsine oxide* slowly separates in quantitative yield in colourless plates, m. p. 131°. The *trans*-isomer prepared in a similar manner has m. p. 143°.

Determination of cis-2-*Chlorovinyldichloroarsine.*—The dichloroarsine (0·4598 g.) was dissolved in chloroform (20 c.c.) and shaken with cold sodium hydroxide solution (4N, 20 c.c.). The aqueous solution was washed twice with chloroform (20 c.c. each time), made just faintly acid to Congo-red, diluted to 300 c.c., and titrated with iodine solution using starch as an indicator. Provided the acid concentration is kept very low, the end point is reasonably sharp. The titre for pure *cis*-isomer was 41·4 c.c. of 1·05N-iodine which gives 98%. The chloroform washings had zero iodine titre. This method is suitable for estimating the *cis*-isomer in the presence of the *trans*-isomer, arsenic trichloride, 2 : 2′-dichlorodivinylchloroarsine, and 2 : 2′ : 2″-trichlorotrivinylarsine, even when all are present in solution, since the *trans*-isomer is converted into sodium arsenite by sodium hydroxide solution and does not therefore react with iodine after acidification. The other materials are insoluble in sodium hydroxide solution and are extracted by the chloroform.

Attempted Isomerisations.—(*a*) Pure *trans*-2-chlorovinyldichloroarsine (55 g.) (prepared by reduction of the pure *trans*-arsonic acid in hydrochloric acid solution) was dissolved in carbon disulphide (250 c.c.), and aluminium chloride (35 g.) added slowly with cooling. After ½ hour in ice and 24 hours at room temperature, the clear layer was decanted and decomposed with ice–hydrochloric acid. Distillation yielded arsenic trichloride (4·5 g.) and *trans*-2-chlorovinyldichloroarsine (9·2 g.). The aluminium chloride sludge after decomposition with ice and hydrochloric acid yielded a mixture of 2 : 2′-dichlorodivinylchloroarsine and 2 : 2′ : 2″-trichlorotrivinylarsine (8·7 g.); there was considerable high-boiling residue.

(*b*) The pure *trans*-isomer (prepared from the arsonic acid) was irradiated in an open dish with light from a mercury vapour lamp; after 24 hours the whole of the dichloroarsine had been converted into the *trans*-arsonic acid. Irradiation in a closed vessel at 1 ft. from the light source gave a product which was mainly the product of disproportionation, but some isomerisation had also occurred as was shown by analysis (arsenic trichloride, 16·1%; *trans*-isomer, 54·6%; *cis*-isomer, 8·5%; 2 : 2′-dichlorodivinylchloroarsine, 19·8%). The arsonic acid was recovered unchanged after irradiation.

(*c*) The *trans*-arsonic acid (100 g.) was treated with phosphorus oxychloride (170 g.) in small amounts with cooling; the product was then distilled. Fraction (i), b. p. 55° (7 g.); (ii), b. p. 100—110° (35 g.); (iii), b. p. 120—130° (88 g.); (iv), b. p. 35—75°/6 mm. (35 g.). Fraction (i) was a mixture of *cis*- and *trans*-dichloroethylene; (ii) was unchanged phosphorus oxychloride; (iii) was arsenic trichloride containing some tetrachloroethane; and (iv) was tetrachloroethane and 2-chlorovinyldichloroarsine. Fraction (iv) on refractionation gave 11 g. of tetrachloroethane and 24 g. of 2-chlorovinyldichloroarsine, b. p. 85—86°/11 mm., which contained only a small amount of *cis*-isomer as determined by above method.

Preliminary Investigation.—2-Chlorovinyldichloroarsine (2400 g.) of boiling range 40—65°/4 mm., prepared by using a cuprous chloride catalyst, was oxidised by stirring with water and passing in chlorine. Solvent and arsenic chloride were removed under reduced pressure and most of the *trans*-arsonic acid removed by crystallisation. The liquors were reconverted into the dichloroarsines and distilled into three fractions: (i) 40—48°/3 mm. (180 g.); (ii) b. p. 48—60°/3 mm. (283 g.); (iii) b. p. > 60°/3 mm. (81 g.). Each fraction was oxidised with water and chlorine. Fraction (i) yielded only 15 g. of *trans*-acid; fraction (ii) yielded 253 g. of the *trans*-acid. Thus the *cis*-isomer is the lower-boiling. The mother liquors of the *trans*-acid from fraction (i) on reconversion into the dichloroarsine gave a product which liberated only 28% of the theoretical amount of acetylene with cold sodium hydroxide solution.

The author wishes to thank Mr. H. W. Vallender for valuable assistance in this work and the Chief Scientist of the Ministry of Supply for permission to publish it.

Chemical Defence Research Establishment,
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[*Received, February 4th*, 1948.]

238. *A Determination of the Structures of the Isomeric 2-Chlorovinyldichloroarsines.*

By C. A. McDowell, H. G. Emblem, and E. A. Moelwyn-Hughes.

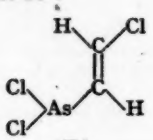
By measuring the dipole moments of the isomeric forms of 2-chlorovinyldichloroarsine in benzene solution, it has been established that ordinary "lewisite" is *trans*-2-chlorovinyldichloroarsine, while the new isomer isolated by Hewett (preceding paper) and termed "*iso*lewisite", has the *cis*-configuration.

Classical stereochemical methods have generally been decisive in elucidating the configurations of optically active molecules, but they have not been so successful in cases of geometrical isomerism. Most of the purely chemical methods of attacking the latter type of problem depend on the assumption of *cis*- or *trans*-addition of reagents or elimination of groups, and it is only recently that evidence has been accumulated which shows that *trans*-addition and elimination are the processes which usually occur. In fact, even with this knowledge it is rarely possible to prove the configuration of *cis*- or *trans*-isomers by purely chemical methods alone. It is, however, often possible to supply strong supporting, and sometimes conclusive, evidence by the application of some of the techniques of physical chemistry.

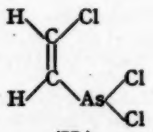
Of the various methods which might be used for this purpose, dipole-moment measurements are usually the easiest to carry out, and often give a unique answer. It is, in general, possible to estimate the probable dipole moment of a compound from its structure, by vectorial addition of the moments of the individual linkages. Comparison of this theoretical moment with the observed value shows whether or not the assumed structure is correct. This is the most satisfactory way of using dipole-moment measurements for structural determinations; but, in those cases of *cis–trans* isomerism where the groups in the molecule have approximately the same moment, it is often assumed that the compound with the larger moment is the one with the *cis*-configuration. In this way dipole moment measurements have helped to establish the configurations of the *cis*- and *trans*-isomers of the dihalogenoethylenes (Errera, *Compt. rend.*, 1926, **182**, 1623; *J. Phys. Radium*, 1925, **6**, 390; *Physikal. Z.*, 1926, **27**, 764), 2 : 2′-azoxytoluene and 2 : 2′-azoxyanisole (Müller, *Annalen*, 1932, **495**, 132), benzenediazocyanides (Le Fèvre and Vine, *J.*, 1938, 431), and azobenzene (Hartley and Le Fèvre, *J.*, 1939, 531); while similar measurements on the *O*- and *N*-methyl ethers of *p*-nitrobenzophenone oxime and the *O* ether of the corresponding aldoxime have enabled Sutton and Taylor (*J.*, 1931, 2190; Taylor and Sutton, *J.*, 1933, 63) to show that the Beckmann rearrangement takes place by a *trans*-interchange of the migrating groups.

Hewett (previous paper) has described the preparation of isomeric 2-chlorovinyldichloroarsines and the corresponding arsonic acids, and has shown that, though the two isomers have different physical and chemical properties, it is not possible by chemical evidence alone unambiguously to assign a configuration to either. On the basis of the available evidence it is assumed that the isomer which is formed in larger amounts, "lewisite", has the *trans*-configuration (I), and that the other more volatile isomer, "*iso*lewisite", has the *cis*-configuration (II). The main chemical difference between these two substances is their behaviour on treatment with cold dilute sodium hydroxide solution. Lewisite readily yields acetylene, whereas *iso*lewisite dissolves in the sodium hydroxide without evolution of gas, but on warming to 40° yields vinyl chloride.

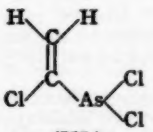
With a view to obtaining more conclusive proof of the above assignments, and also to rule out the possibility that *iso*lewisite may be not a geometrical isomer, but the position isomer 1-chlorovinyldichloroarsine (III), we have measured the dipole moments of these substances in benzene at 25°.



(I.)



(II.)



(III.)

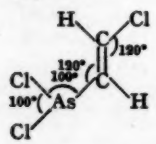
The dipole moment of lewisite was found to be $2{\cdot}20 \times 10^{-18}$ e.s.u. which is higher than the value 1·77 D. given by Möhler (*Helv. Chim. Acta*, 1938, **21**, 789). Möhler gives but few physical constants for the lewisite which he used, so we have no means of comparing the purity of our respective samples. It should be noted, however, that Möhler gives the electronic polarisation,

P_E, as 43·2 c.c. whereas the value calculated from our determinations of the density and refractive index of lewisite is 38·1 c.c. It should be pointed out that our values for the density and refractive index of lewisite are in very close agreement with the standard values for the vesicant as determined by the Chemical Defence Research Establishment. The difference in the two values for the dipole moment of lewisite is rather larger than is usually found for such measurements and must, therefore, be considered greater than the experimental error. At first it was thought that our lewisite was contaminated with some iodo-analogue, for, in its preparation, 2-chlorovinylarsonic acid is reduced with sulphur dioxide in the presence of a trace of iodine (Hewett, *loc. cit.*). An iodine-free sample was, therefore, obtained by carefully purifying crude lewisite prepared by the action of acetylene on arsenic trichloride in the presence of cuprous chloride (Jones *et al.*, *Ind. Eng. Chem.*, in the press); its dipole moment proved to be 2·23 D., in good agreement with that obtained for the previous sample. We have, therefore, adopted 2·21 D. as being the most reasonable value for the dipole moment of ordinary lewisite in benzene at 25°. The dipole moment of the *iso*lewisite isolated by Hewett (*loc. cit.*) was next measured under the same conditions and was found to be 2·61 D. The difference between the dipole moments of lewisite and *iso*lewisite, $\Delta\mu = 0{\cdot}4$ D., is of the same order of magnitude as the difference in the moments of the *O*-methyl ethers of the *cis–trans* isomeric *p*-benzophenone oximes ($\Delta\mu = 0{\cdot}51$ D., Taylor and Sutton, *loc. cit.*) and of the *O*-methyl ethers of similar isomeric *p*-benzaldoximes ($\Delta\mu = 0{\cdot}49$ D., *idem*, *ibid.*). It thus seems reasonable to assume that lewisite is *trans*- and *iso*lewisite is *cis*-2-chlorovinyldichloroarsine. It has still, however, to be shown that *iso*lewisite is not 1-chlorovinyldichloroarsine. This possibility can be eliminated, and the evidence for the above assignments strengthened, by comparing the experimental values of the dipole moments with those calculated for structures (I), (II), and (III), from known, or estimated, contributions of the different linkages in these molecules.

The $=C<^{H}_{Cl}$ group moment has been taken as being equal to the dipole moment of vinyl chloride, $\sim 1{\cdot}4$ D., in benzene solution (the dipole moment of vinyl chloride in gas phase is 1·44 D. (Hugill, Coop, and Sutton, *Trans. Faraday Soc.*, 1938, **34**, 1518); and the C–As bond calculated from the dipole moment of $AsPh_3$ to be 1·07 D. (Bergmann and Schütz, *Z. physikal. Chem.*, 1932, *B*, **19**, 401). The moment of the $AsCl_2$ group was calculated from the dipole moment of arsenic trichloride, $\mu = 2{\cdot}17$ D. (Smythe, *Proc. Roy. Soc.*, 1932, *A*, **136**, 256), knowing that this molecule is pyramidal with $\measuredangle$ Cl–As–Cl $\simeq 100°$ (Pauling and Brockway, *J. Amer. Chem. Soc.*, 1935, **57**, 2684); from these data we get $\mu_{AsCl_3} = 1{\cdot}99$ D. Using these estimated contributions of the linkages and groups and the valency angles assumed in the following structures (Brockway, Beach, and Pauling, *ibid.*, p. 2693), Dr. L. E. Sutton has estimated, at our request, the values shown for the dipole moments of these compounds; he writes: "The theoretical values for the moments of these compounds, on the assumption that rotation of the asymmetrical polar groups about single bonds is completely free, were calculated by means of the formula

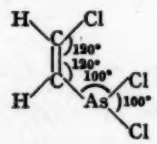
$$\bar{\mu}^2 = \mu_S^2 + \mu_R^2$$

where $\bar{\mu}$ is the average moment, μ_S is the resultant moment of all the non-rotating components, and μ_R is the rotating component (see Fuchs and Wolf, "Dielektrische Polarisation", Leipzig, 1935, p. 397)."



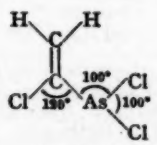
(I*a*.)

trans-2-Chlorovinyldichloroarsine
$\mu_{calc.} = 2{\cdot}03$ D.



(II*a*.)

cis-2-Chlorovinyldichloroarsine
$\mu_{calc.}$ { = 2·14 D. (no rotation of $AsCl_2$ group)
= 3·2 D. (free rotation of $AsCl_2$ group)



(III*a*.)

1-Chlorovinyldichloroarsine
$\mu_{calc.} = 1{\cdot}15$ D.

On comparing these theoretical estimates with the experimentally determined values of the dipole moments of lewisite and *iso*lewisite we can immediately eliminate 1-chlorovinyldichloroarsine as a possible structure. Further, we see that the isomer with the smaller dipole moment is obviously *trans*-2-chlorovinyldichloroarsine whilst *iso*lewisite is *cis*-2-chlorovinyldichloroarsine. It will be noted that the experimentally observed dipole moment of *iso*lewisite lies between the values calculated for *cis*-2-chlorovinyldichloroarsine with and without free rotation

of the $AsCl_2$ group, and is consistent with a hindered rotation. Considering the approximate nature of these estimations the agreement with experiment is very satisfactory and leaves little doubt that the assigned configurations are correct.

Experimental.

The experimental method and apparatus used have been described by Emblem and McDowell (*J.*, 1946, 641).

Materials.—Benzene. " AnalaR " Benzene was washed with concentrated sulphuric acid, dried over sodium, distilled, and frozen thrice to remove homologues. A sample was refluxed over phosphoric oxide for about 10 hours, stored over this reagent, and distilled from it immediately before use. It had $n_D^{20°}$ 1·5010.

Lewisite. Two specimens of lewisite were used. Specimen *A* was prepared from the corresponding arsonic acid by reduction with sulphur dioxide in the presence of a trace of iodine (Hewett, *loc. cit.*), and had $n_D^{20°}$ 1·6105, $d_4^{20°}$ 1·8877. Specimen *B* was the specially prepared iodine-free lewisite obtained by purification of the plant product and had $n_D^{20°}$ 1·6106, $d_4^{20°}$ 1·8873.

iso*Lewisite.* This was a sample of the pure isomer prepared by Hewett (*loc. cit.*) and had $n_D^{20°}$ 1·5888, $d_4^{20°}$ 1·86827.

Results.—In the following tables ϵ = dielectric constant of the solution; w_1 = weight fraction of solute; d = density of solution; p_{12} = specific polarisation of solution; P_1 = molar polarisation of solute in benzene solution. The specific and molar polarisations were calculated by Sugden's method (*Trans. Faraday Soc.*, 1934, **30**, 722). All values are at 25°.

Molar polarisation of solutions of lewisite (specimen A) in benzene.

w_1.	d.	ϵ.	p_{12}.	P_1.
0·01565	0·8777	2·3087	0·34606	139·54
0·06307	0·9015	2·4513	0·36166	139·02
0·10160	0·9216	2·5710	0·37293	136·1
0·12326	0·9335	2·6464	0·37958	135·78

By graphical extrapolation of these data we get the molar polarisation at infinite dilution P_∞ = 140 c.c. From the refractive index and density of this specimen we get the electronic polarisation, P_E to be 38 c.c. Taking the atomic polarisation P_A to be 5% of P_E as suggested by Sugden and Groves (*J.*, 1937, 1779, 1782) we get P_{A+E} = 40 c.c.; hence, using the equation $P_\infty - P_{E+A} = P_0$, we get P_0, the orientation polarisation, to be 100 c.c.; this yields for the dipole moment of lewisite in benzene solution at 25° the value $2{\cdot}20 \times 10^{-18}$ e.s.u.

Molar polarisation of solutions of lewisite (specimen B) in benzene.

w_1.	d.	ϵ.	p_{12}.	P_1.
0·02368	0·8831	2·3368	0·34905	142·37
0·05115	0·8989	2·4247	0·35820	140·95
0·0688	0·9060	2·4750	0·36382	139·83
0·1451	0·9475	2·7268	0·38556	134·51

Graphical extrapolation of these data yields P_∞ = 143·2 c.c. Since P_{E+A} = 40 c.c., we get P_0 = 103·2 c.c.; hence $\mu = 2{\cdot}23 \times 10^{-18}$ e.s.u. The agreement between these two values for the dipole moment of the two different specimens of lewisite is very satisfactory, and in this paper we shall adopt $\mu = 2{\cdot}21 \times 10^{-18}$ e.s.u. as being the best value for this constant at 25°.

Molar polarisation of solutions of iso*lewisite in benzene.*

w_1.	d.	ϵ.	p_{12}.	P_1.
0·04710	0·8948	2·4517	0·36445	174·49
0·0565	0·8997	2·4898	0·36882	173·24
0·06636	0·9052	2·5325	0·37351	172·68
0·1173	0·9329	2·7519	0·39519	166·69

From these data we obtain by graphical extrapolation P_∞ = 180 c.c. The refractive index and density of *iso*lewisite yield P_E = 37·4 c.c.; hence we take P_{E+A} as 39·28 c.c. This gives for P_0 the value 140·72 c.c., from which we calculate the dipole moment, μ, of *iso*lewisite in benzene solution at 25° to be $2{\cdot}61 \times 10^{-18}$ e.s.u.

Pure specimens of lewisite and *iso*lewisite were prepared, respectively, by Dr. R. L. Barker and Dr. C. L. Hewett. We are indebted to Dr. L. E. Sutton for helpful advice and for permission to incorporate his theoretical estimates of the moments of the isomeric 2-chlorovinyldichloroarsines in this paper. Dr. G. H. Whiting kindly supplied information concerning the physical constants of both liquids. The work described here was carried out in the Chemical Defence Research Establishment of the Ministry of Supply, and is published with the consent of its Chief Scientist.

Department of Physical and Inorganic Chemistry,
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[*Received, May 28th*, 1947.]

239. *Some Physicochemical Properties of* cis-2-*Chlorovinyldichloroarsine.*

By G. H. Whiting.

The physicochemical constants—freezing point, vapour pressure, density, refractive index, and viscosity—for *cis*-2-chlorovinyldichloroarsine have been determined and compared with the data for the *trans*-isomer.

The substance of which the isolation and configuration are described in the two preceding papers is characterised by the following measured and derived physicochemical constants, which can be compared (col. 3) with those of the more abundant *trans*-isomer.

Table I.

Physicochemical constants of cis- *and* trans-2-*chlorovinyldichloroarsine.*

Property.	*cis*-Isomer.	*trans*-Isomer.
Freezing point	−44·7°	−1·2°
Vapour pressure (mm. Hg) at 25°	1·562	0·40
log p (mm. Hg)	8·4131—2450·2/T	48·660—13·297 log T −4815·3/T
B. p./760 mm.	169·8°	196·6°
Latent heat of vaporisation at 25°, $L_V^{25°}$ (cal./g.-mol.)	11,220	15,150
L_V at the b. p.	11,220	9,620
Molar b. p. depression	34·7°	45·6°
$d_4^{25°}$	1·8598	1·8793
$d_4^{t°}$	1·9018—0·00168t	1·9210—0·00167t
$n_D^{25°}$	1·5859	1·6076
$n_D^{t°}$	1·6003—0·000575t	1·6201—0·0050t
$[R_L]_D$	37·388	38·089
η at 25° (g./cm.-sec.)	0·0169	0·0205
log η	590/T − 3·751	699/T − 4·033

Experimental.

Purity.—The purest material tested (Preparation III, supplied by Dr. Hewett) consisted of 17 ml. of a liquid which froze continuously and completely during 20 minutes at − 44·7°, constant to ± 0·05°. The 3-ml. sample first prepared (Dr. Hewett's Preparation I) froze at 2·0° lower; whilst Preparations IV, V, etc. (after extraction with benzene and distillation), also froze at a lower temperature, besides showing some deviation in density and refractive index. In view of the prevailing possibility of disproportionation to arsenic trichloride and dichlorovinylchloroarsine (see Hewett, this vol., p. 1203), the above-mentioned freezing behaviour may be taken as a satisfactory test of the purity of Preparation III. The consistent and reproducible vapour-pressure relations of Preparation III support that assumption.

Freezing Point.—In order to obtain a uniform and slow rate of cooling of small specimens, the apparatus shown in Fig. 1 was used. The substance under investigation was contained in a small jacketed tube *A* just large enough to hold a 1-mm. layer of liquid around the bulb of a calibrated thermometer, reading to 0·1° and rigidly held by a clamp. The envelope of tube *A* was surrounded by a cooling bath *B* of alcohol and solid carbon dioxide, held several degrees below the temperature of the thermometer; and any desired rate of cooling was obtained by appropriate evacuation of the jacket through the side arm and tap *C*. Agitation was effected by moving *A* against the tension of a piece of elastic *D*, and seeding was done (in dry nitrogen) by introducing nuclei from a subsidiary frozen sample in the same bath, on the end of thin glass needles. The time–temperature graphs obtained by reading the temperature every 10—30 seconds gave unmistakable indications, both of the freezing point and of the degree of impurity, for every sample tested.

Vapour Pressure.—The vapour pressure was determined by distillation of the liquid from a heated "Pyrex" bulb of 50 ml. capacity into another bulb of the same capacity in a freezing mixture against several arbitrary pressures established in the system after thorough evacuation. The distillation temperatures were measured by a calibrated thermometer, in a mercury pocket projecting into the distillation stream, whilst the pressures were measured on a mercury manometer, and reduced to mm. of mercury at 0°. The mid-fractions of five distillations yielded 16 points on a smooth curve, and the corrected readings, given in Table II, were used for calculating the equation in Table I by the method of least squares. The points agreed with the formula to an average deviation of 2% on the pressure readings.

Table II.

Vapour pressure of cis-2-*chlorovinyldichloroarsine.*

	p (mm. Hg).			p (mm. Hg).			p (mm. Hg).			p (mm. Hg).	
Temp.	Obs.	Calc.	Temp.	Obs.	Calc.	Temp.	Obs.	Calc.	Temp.	Obs.	Calc.
67·6°	16·9	16·3	76·8°	26·1	25·7	85·9°	39·1	38·7	100·1°	70·7	71·0
72·2	19·9	20·8	78·0	27·7	27·2	87·1	39·8	40·8	102·6	76·2	77·9
72·4	20·7	21·0	80·9	31·3	31·0	91·2	49·1	48·7	107·1	92·6	89·5
76·8	26·2	25·7	85·3	37·5	37·7	94·7	56·6	56·4	109·3	100·0	101·3

Density.—This was determined in duplicate in a type of pycnometer shown in Fig. 2. It consisted of a hemispherical "Pyrex" bulb of 10-ml. capacity, fitted with two capillary necks (of 0·2 mm. internal diameter, and graduated in mm.), the lower of which (*A*) was provided with a grooved stopper as shown. Filling was effected through the capillary *A* from a burette at atmospheric pressure. The true volume of the pycnometer, at any temperature, and measured to any graduations, was obtained by previous calibration with distilled water, all the usual precautions being used. The figures obtained agree with the formula in Table I to ± 0·0001 :

Density of cis-2-*chlorovinyldichloroarsine.*

Temp.	d_4^t.	Temp.	d_4^t.	Temp.	d_4^t.
20·00°	1·86831, 1·86821	25·00°	1·85980	30·00°	1·85149

Refractive Index.—The refractive index was determined in an Abbé refractometer, adjusted to a standard prism, and checked with distilled water. Readings were taken when the temperature of water circulating from a thermostat had been constant to 0·1° for ten minutes. The readings obtained on rising and falling temperature sequences agree with the formula in Table I to ± 0·0002.

FIG. 1.

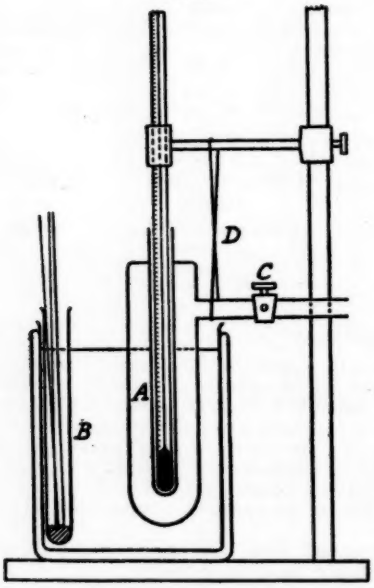
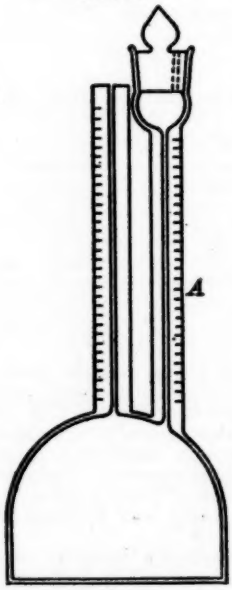


FIG. 2.



Refractive index of cis-2-*chlorovinyldichloroarsine.*

Temp.	21·0°	25·0°	30·0°	35·0°	37·0°	27·0°	23·0°	20·0°
n_D^t	1·5882	1·5860	1·5832	1·5802	1·5791	1·5848	1·5869	1·5888

Viscosity.—The viscosity of the liquid was determined in a BSS Ostwald-type viscometer in a thermostat steady to 0·1° at temperatures from 20° to 35°, and in dried air. The results obtained agree with the formula in Table I to 0·001 in log η in all cases but one.

Viscosity of cis-2-*chlorovinyldichloroarsine.*

Temp.	20·0°	25·0°	30·0°	35·0°
η (g./cm.-sec.)	0·01831	0·01690	0·01561	0·01461

Data for the trans-*Isomer.*—The comparable data for *trans*-2-chlorovinyldichloroarsine, given in Table I, form part of a comprehensive review of standard data for the physicochemical properties of toxic materials, which was conducted at the Chemical Defence Research Establishment during the last war, and which it is hoped to publish in due course.

The author wishes to thank Dr. C. L. Hewett for the isolation and purification of the materials tested, and his colleagues C. A. MacDowell, H. G. Emblem, J. F. Sumner, S. Cook, and S. W. Matheson for help in the experimental work. Grateful acknowledgment is also made to Dr. E. A. Moelwyn-Hughes, under whose direction the work was carried out.

This paper is published with the approval of the Chief Scientist, Ministry of Supply.

CHEMICAL DEFENCE RESEARCH ESTABLISHMENT,
SUTTON OAK.

[*Received, August 11th,* 1947.]

240. *The Constitution of a Specific Somatic Polysaccharide from* M. tuberculosis (*Human Strain*).

By Sir Norman Haworth, P. W. Kent, and M. Stacey.

Two stable serologically specific polysaccharide fractions (having $[\alpha]_D^{18°}$ + 85° and $[\alpha]_D^{18°}$ + 25° in water respectively) together with a deoxyribonucleic acid derivative and bacterial glycogen have been isolated from heat-killed cells of *M. tuberculosis* (human strain).

The specific polysaccharide having $[\alpha]_D^{18°}$ + 85° was shown to originate mainly from the somatic part of the cell, while the specific polysaccharide having $[\alpha]_D^{18°}$ + 25° was shown to occur in close association with the cell lipoids.

The specific somatic polysaccharide was smoothly converted into its methyl ether, hydrolysis of which yielded 3 : 5-dimethyl methyl-D-arabofuranoside (21·9%), 3 : 4 : 6-trimethyl methyl-D-mannopyranoside (26·6%), 3 : 4-dimethyl methyl-D-mannopyranoside (20·2%), 2 : 3 : 4-trimethyl methyl-L-rhamnopyranoside (24·8%), and a methylated amino-sugar derivative (5·8%). This polysaccharide thus has a highly branched structure composed of mannopyranose, arabofuranose, and amino-sugar units with rhamnopyranose units forming terminal residues. Osmotic pressure measurements indicated a molecular weight of approximately 12,000.

For the first time D-arabofuranose was conclusively identified as a component of a naturally-occurring substance.

Complex carbohydrates of *M. tuberculosis* have been isolated from three sources, namely, from the somatic portion of the cell, from the cell lipoids, and from the culture medium.

Laidlaw and Dudley (*Brit. J. Exp. Path.*, 1925, **6**, 197) separated a specific somatic polysaccharide, glycogen, and nucleic acid by the action of dilute acid upon heat-killed organisms. The specific polysaccharide ($[\alpha]_D$ + 67°) reacted with serum from tuberculous animals in high dilution, and the authors reported that it gave positive tests for pentose and methyl pentose.

A similar dextrorotatory ($[\alpha]_D$ + 72°) specific polysaccharide was obtained by Maxim (*Biochem. Z.*, 1930, **223**, 404).

Heidelberger and Menzel (*Proc. Soc. Exp. Biol. Med.*, 1932, **29**, 631) separated a number of fractions by acid extraction of autolysed cells. It was shown that the principal serologically active fraction ($[\alpha]_D$ + 85°) contained D-arabinose and D-mannose. These authors (*J. Biol. Chem.*, 1937, **118**, 79; 1939, **127**, 221) found also a second specific somatic polysaccharide having a low dextrorotation.

The presence of D-galactose in polysaccharides of *M. tuberculosis* was shown by Gough (*Biochem. J.*, 1932, **26**, 248). Although the polysaccharides exhibited weakly acidic reactions, no glycuronic acid constituent could be detected among the products of hydrolysis.

Two serologically active polysaccharides were isolated by Chargaff and Schaefer (*J. Biol. Chem.*, 1935, **112**, 393) who showed that both contained D-arabinose and D-mannose, and reported for the first time that one contained a small amount of glucosamine.

Pentose-containing polysaccharides in the filtered culture medium were investigated by Meuller (*J. exp. Med.*, 1926, **43**, 9) who showed that some fractions had high serological activity.

The separation of polysaccharide from protein components was achieved electrophoretically by Seibert (*ibid.*, 1938, **68**, 413; *J. Biol. Chem.*, 1941, **140**, 55) who demonstrated the presence of at least two polysaccharides in the culture medium. One of these was a serologically active, nitrogen-containing substance with a molecular weight of 9000 (cf. the value of 7300 found by Tennet and Watson, *J. Immunol.*, 1942, **45**, 179).

In 1930, Masucci, McAlpine, and Glenn (*Amer. Rev. Tuberc.*, 1930, **22**, 699, 678) isolated a polysaccharide, $[\alpha]_D$ + 67°, of high serological activity from the culture medium, and showed that it contained D-arabinose and D-mannose.

Polysaccharides have been derived from a third source in *M. tuberculosis*, namely from the lipoid constituents. A systematic study of this group has been made by Anderson and his co-workers (*Chem. Reviews*, 1941, **29**, 225). From the phosphatide fraction a trisaccharide containing mannose and inositol was isolated, and in the acetone-soluble fats there were constituents which appeared to be esterified with trehalose.

The investigation showed that the principal lipoid component of the cells was a wax-like substance which, on alkaline hydrolysis, yielded a mixture of fatty acids and serologically active polysaccharide in which D-mannose, D-galactose, D-arabinose, and a trace of glucosamine were identified. The polysaccharide ($[\alpha]_D$ + 30°) reacted with immune serum in a high dilution (cf. following paper).

In spite of the immense number of polysaccharide fractions of seemingly divergent properties,

there appear to be two main fractions throughout. Both contain traces of nitrogen and phosphorus, D-arabinose, and D-mannose, and one has a lower pentose content and higher dextrorotation than the other.

No structural studies of any of the polysaccharides described have so far been attempted. It was the purpose of this present work to prepare relatively stable specific polysaccharide fractions in an essentially homogeneous form and to elucidate as far as possible some details of their configuration in order to gain information on possible immunising complexes of the tubercle bacillus.

In an extended search for high molecular complexes which might possess wider immunological properties than those of the hapten polysaccharides, there was obtained from a urea extract of heat-killed cells of *M. tuberculosis* a compound which contained a polysaccharide, $[\alpha]_D^{18°} + 28°$, in close association with deoxyribonucleic acid and lipoids, including the " acid-fast " mycolic acid.

This polysaccharide, the " lipoid-bound " polysaccharide, described in the following paper, could be isolated from the complex by means of alkaline hydrolysis, and fractionation methods showed it was essentially homogeneous. This lipoid-bound polysaccharide reacted with tuberculosis anti-serum in high dilution (1 : 2,000,000) when submitted to the precipitin test. When the main lipoid constituents had been removed by means of urea from heat killed *M. tuberculosis* cells, there remained the somatic portion of the cells from which on strong alkaline hydrolysis a second specific polysaccharide the " somatic " polysaccharide, $[\alpha]_D^{18°} + 85°$, was isolated.

Isolation of the Polysaccharides.—As a first approach to the problem of procuring relatively large quantities of both polysaccharides, it was considered that stable fractions, which would not readily undergo further degradation, could be obtained on alkaline hydrolysis of the whole cells so that by subsequent fractionation methods, using chiefly precipitation from solvents, reasonably homogeneous material might be obtained.

In view of the high lipoid content of the cells, as much fatty material as possible was first removed with organic solvents (ether, acetone, etc.), and then the dry partly defatted residue was warmed with alkali which served to liberate the more firmly bound lipoids and enabled the polysaccharides to pass into solution. The solution containing the polysaccharide was acidified, and some insoluble matter separated off on the centrifuge. The liquid was then freed from inorganic and other low-molecular impurities by dialysis. To the resulting solution were added several volumes of alcohol, and the crude polysaccharide thus thrown down was again dialysed and reprecipitated from an acid solution by addition of alcohol or acetone. The initial polysaccharide mixture was dextrorotatory, contained small amounts of nitrogen and phosphorus, and had weak acidic properties. It gave also a positive Dische test (Dische, *Z. Mikrochem.*, 1910, **8**, 4) for deoxyribonucleic acid, the presence of which or a derivative thereof was confirmed by an absorption band at 2650 A.

It was clear at this stage that the crude product was a mixture of various substances.

Purification. In a typical separation the polysaccharide mixture was resolved into various constituents by fractional precipitation from an aqueous solution with alcohol. Ten initial fractions were separated the first of which was composed mainly of inorganic matter. The second and third fractions contained a substance which imparted a reddish-brown or purple coloration with iodine and which closely resembled the glycogen isolated by earlier workers (Chargaff and Schaefer, 1935, *loc. cit.*; Heidelberger and Menzel, 1937, *loc. cit.*). The three fractions which then separated had essentially similar properties, appeared to be fairly homogeneous, were free from glycogen, and contained only traces of nucleic acid. The somatic polysaccharide was present in almost pure state in these first precipitates ($[\alpha]_D + 80°$).

A mixed fraction was next separated, and finally another three fractions, having similar properties, were separated; these ($[\alpha]_D + 25°$) were mainly composed of a second polysaccharide, which was identical with the lipoid-bound polysaccharide. This again gave a strong test for deoxyribonucleic acid and also had a strong absorption band (at 2650 A.).

The somatic polysaccharide was refractionated by alcoholic precipitation from an aqueous solution. The sub-fractions obtained all had closely similar properties though a trace of deoxyribonucleic acid still remained throughout. This impurity was not precipitated by addition of lanthanum acetate or acetic acid, and it was probable therefore that a deoxyribonucleoside was a contaminant. It was finally removed as described later during the course of methylation and fractionation of the methylated derivatives.

In the case of the lipoid-bound polysaccharide, the nucleic acid derivative was removed by passing an aqueous solution of the fraction repeatedly through ion-exchange resins. The

purified product was recovered in good yield by alcoholic precipitation of the acidified final solution.

Examination of both polysaccharides revealed that the weak acidity was correlated with the nitrogen and phosphorus contents and was doubtless due to the presence of a trace of a nucleic acid derivative. Preliminary qualitative examination showed that both polysaccharides contained a pentose and a small amount of an amino-sugar, and that both products were readily hydrolysed by dilute acids to give a mixture of reducing sugars.

Nature of the Somatic and Lipoid-bound Polysaccharide Fractions.—The place of origin of the two polysaccharide fractions in the bacterial cell was further studied by exhaustive removal of the ether-soluble lipoids from the cells. These cells were extracted with warm dilute alkali, and the polysaccharide isolated from this solution consisted almost entirely of the somatic fraction (above). On the other hand saponification of the numerous samples of the lipoidal constituents of the cell, separated by various methods, afforded only a polysaccharide having properites closely resembling those of the polysaccharide originally isolated, and in no case was any material having a high dextrorotation isolated from the lipoidal " capsular " material. It was thus confirmed that the polysaccharide having $[\alpha]_D + 85°$ is mainly of somatic origin, and that the polysaccharide having $[\alpha]_D + 25°$ is associated with the lipoids which occur in what is termed the waxy layer of the cell.

On further prolonged treatment of either of the purified polysaccharides with alkali, no changes could be detected and the fractions were recovered intact from solution. Thus it is considered that these two substances represent alkali-stable components of the specific polysaccharides of the cell. Further, when both fractions were separately submitted to the precipitin test with human tuberculous antisera, they reacted in a dilution as high as 1 : 2,000,000. This result indicates the specific nature of the polysaccharide fractions, and although, owing to the vigorous mode of their isolation, some degradative changes were likely, the fact is established that part at least of those portions of the molecule responsible for the reaction with immune serum are relatively alkali-stable. In view of this and of the fact that the fractions do not undergo detectable changes on prolonged treatment with alkali, it is reasonable to suppose that this part of the structure of the polysaccharide molecule was not appreciably modified during the subsequent process of methylation described herein.

Methylation of the Somatic Polysaccharide.—The polysaccharide was smoothly methylated by methyl sulphate in the presence of sodium hydroxide. The product had a methoxyl content of *ca.* 40% after six treatments, and this value was not increased by further methylations. The methylated derivative, obtained in the form of a brown gum, was purified by repeated fractional precipitation from organic solvents. The principal fractions had essentially identical properties, *i.e.*, methoxyl content (39·2%), optical rotation $[\alpha]_D^{18°} + 125°$ in chloroform, a very low viscosity, nitrogen, 0·3%, phosphorus content, nil.

A typical sample of the main bulk of the fractionated methylated polysaccharide was readily hydrolysed by means of methanolic hydrogen chloride, and the mixture of glycosides so obtained was separated by distillation in a high vacuum into a series of fractions. The still-residue was rehydrolysed with methanolic hydrogen chloride and the products fractionally distilled as before. A study of the resulting glycosidic fractions showed that five principal constituents were present and these were characterised as follows :

(*a*) 2 : 3 : 5-Trimethyl methyl-L-rhamnoside, identified by conversion into the free sugar and thence into the anilide, identical with that made under similar conditions from 2 : 3 : 4-trimethyl L-rhamnose.

(*b*) A dimethyl methylpentoside fraction which underwent rapid hydrolysis with N/20-sulphuric acid, a change which suggested the presence of furanose structures. The free sugar was oxidised by bromine to the 1-carboxylic acid which was converted *via* the lactone into 3 : 5-dimethyl D-arabonamide. On further methylation, the dimethyl methylpentoside gave a trimethyl methylpentoside which after hydrolysis was oxidised with bromine water. The product afforded a crystalline specimen of 2 : 3 : 5-trimethyl D-arabonamide.

(*c*) A fraction, having properties corresponding to those of a trimethyl methylhexoside, was converted on further methylation and subsequent hydrolysis into 2 : 3 : 4 : 6-tetramethyl D-mannose from which was formed the anilide. This fraction was hydrolysed by being heated with N-sulphuric acid, and crystalline 3 : 4 : 6-trimethyl D-mannopyranose was isolated.

(*d*) The fourth main constituent was methylated further and hydrolysed to a free sugar which was readily converted into 2 : 3 : 4 : 6-tetramethyl D-mannose anilide. Hydrolysis of this fraction with N-sulphuric acid afforded crystalline 3 : 4-dimethyl D-mannopyranose.

(*e*) A small quantity of an amino-sugar derivative was isolated in the following way :

specimens of the nitrogen-containing glycosidic fractions were mixed and warmed at 100° with 2N-sodium hydroxide for 8 hours in order to remove the *N*-acetyl group. The liquid was neutralised and exhaustively extracted with ether. The ethereal solution, after being dried, was saturated with dry hydrogen chloride, and a syrupy hydrochloride thereby precipitated. This was separated, washed with ether, dried, and *N*-acetylated with methanol and acetic anhydride. A small amount of crystalline product was isolated. The syrupy hydrochloride had a methoxyl content of 32·4% (suggesting dimethyl methylhexosamine hydrochloride). The substance has not yet been identified.

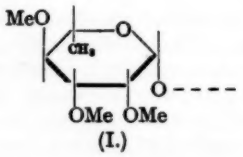
Careful search among the residual syrups gave further small amounts of the above substances but failed to reveal any constituents other than (*a*), (*b*), (*c*), or (*d*).

By reference to the refractive indices and methoxyl contents of the glycosidic fractions a quantitative assay of the amounts of the various constituents was made, the amount of the amino-sugar derivative being calculated also from the nitrogen contents of the various fractions. The following results were obtained: 3 : 5-dimethyl methyl-D-arabinoside (22·0%); 2 : 3 : 4-trimethyl methyl-L-rhamnoside (24·7%); 3 : 4 : 6-trimethyl methyl-D-mannoside (26·8%); 3 : 4-dimethyl methyl-D-mannoside (20·2%); a dimethyl methyl-2-acetamidohexoside (5·9%). This corresponded to a molecular proportion of 25%, 25%, 25%, 20%, and 5% respectively which clearly indicates a molecular ratio of 5 : 5 : 5 : 4 : 1.

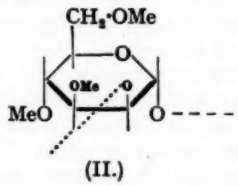
Discussion.

The isolation of a 2 : 3 : 4-trimethyl methyl-L-rhamnoside in 25% yield from the methylated polysaccharide indicates that this sugar can only be linked glycosidically through the position 1, and therefore the whole of the L-rhamnopyranose constituents form end groups linked as in (I).

By the identification of 3 : 4 : 6-trimethyl methyl-D-mannopyranoside it is clear that D-mannopyranose is a constituent unit of the polysaccharide chain and is linked through the 1 and 2 position as in (II). Other mannose units, also in the pyranose form, are linked as in (III) through the 1, 2, and 6 position as deduced from the isolation of 3 : 4-dimethyl methyl-D-



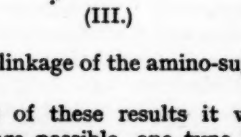
(I.)



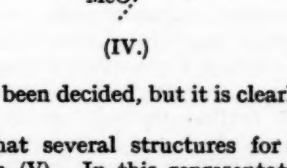
(II.)

mannoside. Since an appreciable amount (20%) of this dimethyl mannose is present the polysaccharide must have a highly branched structure, and this is in keeping with the high percentage (25%) of terminal residues isolated.

The occurrence of 3 : 5-dimethyl methyl-D-arabinose is of considerable importance, since it is the first time that the presence of this rare sugar in the furanose form has been conclusively demonstrated in a substance of biological origin. This pentose must be linked through the 1 and 2 positions as in (IV).



(III.)

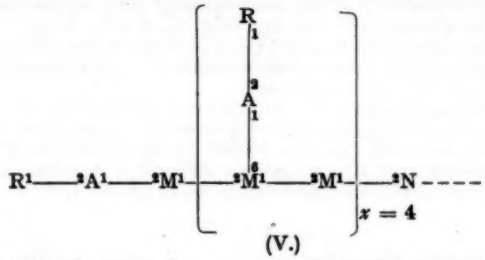


(IV.)

The mode of linkage of the amino-sugar has not yet been decided, but it is clearly not an end group.

In the light of these results it will be seen that several structures for the somatic polysaccharide are possible, one type being shown in (V). In this representation all those linkages engaging the glycosidic groups are temporarily depicted as being in the α-form since the direction of the change of rotation on acid hydrolysis of both the original polysaccharide ($[\alpha]_D + 85° \longrightarrow 22°$) and its methylated derivative ($[\alpha]_D + 125° \longrightarrow + 47°$) is in a negative direction.

Based on the nitrogen content of the methylated polysaccharide it will be seen that if a single amino hexose unit only is present in one molecule of the polysaccharide, then x will be 4.



(V.)

R represents L-rhamnopyranose M represents D-mannopyranose.
A ,, D-arabofuranose. N ,, an amino-hexose.

(The numerals represent the points of attachment.)

The structure of the somatic polysaccharide in (V), elucidated by the methylation technique, represents only the type of the minimum repeating pattern of the entire polysaccharide. The somatic polysaccharide molecule may be any multiple of the suggested structure. In the case of this somatic polysaccharide, that part of the macromolecule shown in (V) may be linked glycosidically through its amino-sugar residue to one constituent of a second similar repeating unit. Extension of this arrangement would indicate more completely the macromolecular structure, information on which might be obtained from osmotic pressure and other physical determinations.

Osmotic Pressure Investigation.—A method for the determination of the molecular weight of substances soluble in organic solvents has been developed by Mr. C. Graff-Baker in our department, based on the earlier findings of Carter and Record (*Chem. and Ind.*, 1936, **55**, 218). A full description of the apparatus and the experimental procedure is to be published later.

Investigation of the osmotic behaviour of chloroform solutions of the fractionated methylated somatic polysaccharide (OMe, 39·2%; $[\alpha]_D$ + 125° in chloroform) indicated that the minimum molecular weight of the methylated derivative was 12,000. The molecular weight of the methylated fraction as derived from (I) is 3898. Thus it is clear that at least 3 such residues are joined together in the intact polysaccharide molecule.

Periodate Oxidation.—Some results kindly obtained for us by Dr. J. K. N. Jones and Mr. T. G. Halsall in Professor Hirst's laboratory are of particular interest. These workers have developed reliable methods for the determination of the number of end-groups in polysaccharide molecules (Hirst *et al.*, *Nature*, 1945, **156**, 785). In the method, the polysaccharide is submitted to oxidation by periodate ions. For each pyranose end group of the polysaccharide, one mole of formic acid is liberated. From the amount of formic acid liberated and the amount of periodate taken up in the oxidation, the number of end-groups can be calculated.

This method has been applied to the somatic polysaccharide. The experimental value, shown below, for the somatic polysaccharide indicates the presence of 4 rhamnose end-groups in every 20 sugar units, as compared with 5 rhamnose end-groups found by the methylation technique and shown in (V).

Thus, accepting the osmotic pressure measurements by which three such structures are shown to be mutually linked (*e.g.*, through the amino sugar and through a rhamnose unit), it is clear that the somatic polysaccharide will have at least 13 end-groups in every 60 sugar units. This is in close agreement with periodic oxidation results.

Experimental.

The cells used consisted of a human strain of *M. tuberculosis* grown on a synthetic medium, and were obtained from the Ministry of Agriculture and Fisheries laboratories (Weybridge), through the courtesy of Dr. Green and his colleagues.

Isolation of a Crude Polysaccharide Mixture.—Moist steam-killed cells (856 g.) were extracted with acetone (1 l.) for 72 hours in a Soxhlet apparatus. The residue was then extracted with ether until no further lipoidal material was removed. Evaporation of acetone and ethereal extracts afforded 31 g. of lipoids.

The dry mainly-defatted cells were warmed with sodium hydroxide (1 l. of N) for 110 hours. The solid matter was separated and the supernatant liquor was acidified and centrifuged. To the liquid so obtained was added alcohol (3 vols.) and the crude polysaccharides removed. This material ($[\alpha]_D^{14°}$ + 36·6°; ash content 31·8%) gave strong Molisch and Bial's (pentose) tests.

The crude product was dissolved in water (250 c.c.), acidified (Congo-red) with dilute hydrochloric

acid, and dialysed in a cellophane membrane. The contents of the membrane were centrifuged and the supernatant liquid treated with alcohol (5 vols.). The product was separated and dried ($[\alpha]_D$ + 85°; 4 g.; ash content 0·6%).

By repetition of this typical experiment, there were obtained 40 g. of starting material which gave positive Molisch, Dische, and phosphate tests and had $[\alpha]_D^{19°}$ + 52·5° in water. This material had an acid equivalent of 2,064, and ash content of 3·9%, and gave a red coloration with iodine.

The ash content could be reduced still further (0·6%) by repeated precipitation from an aqueous acid solution or by prolonged dialysis of an aqueous solution.

Fractionation of the Crude Polysaccharide.—The material was fractionated in quantities of 10 g., each dissolved in 100 c.c. of water. Alcohol was added dropwise to the aqueous solution until a precipitate appeared. This was separated, and more alcohol added to the supernatant liquor until a second precipitate was obtained. Repetition of this procedure afforded 9 fractions, a tenth fraction being isolated by the evaporation of the final alcoholic mother liquors (see Table I).

TABLE I.

No.	Wt. (g.).	$[\alpha]_D$.*	Ash, %.	Bial.	Molisch.	Fehling.	Iodine.	Dische.	Remarks.
1	0·05	+17°	14·9	±	+++	±	Nil	±	—
2	0·13	+61	7·5	+	+++	±	Brown	±	Bacterial glycogen.
3	0·29	+82	3·4	+	+++	—	Reddish-brown	—	—
4	3·00	+85	1·6	++	+++	—	Nil	±	Mainly specific somatic polysaccharide plus traces of nucleic acid derivative.
5	0·89	+79	1·1	++	+++	—	Nil	±	
6	0·28	+96	2·0	++	+++	—	Nil	±	Mixed fractions.
7	0·40	+44	0·5	++	+++	—	Nil	+	—
8	3·75	+28	2·5	++	+++	—	Nil	++	Mainly lipoid-bound specific polysaccharide plus nucleic acid derivative.
9	0·10	+27	1·7	++	+++	—	Nil	+	
10	0·12	+29	1·3	+	+++	—	Nil	+	

* In N/2-sodium hydroxide.

In an attempt to purify the specific polysaccharide, fractions 4 and 5 (above) were dissolved in water (40 c.c.) and reprecipitated by the gradual addition of alcohol. Three sub-fractions were thus obtained (Table II), but these were still contaminated by the deoxynucleic acid.

TABLE II.

No.	Alcohol added (c.c.).	Wt. (g.).	$[\alpha]_D$.	Dische.	Bial.	Iodine.
4*a*	30	0·3	+74°	±	+	Nil
4*b*	80	0·2	+87°	—	±	Brown
4*c*	190	2·35	+85°	±	+	Nil

Investigation of the Nucleic Acid Derivative in the Polysaccharide Fractions, $[\alpha]_D$ + 85° *and* $[\alpha]_D$ + 25°.—(i) The fractions were examined by the Dische test (Dische, *loc. cit.*); fraction 4 gave only a weakly positive reaction whereas the test was strongly positive with fraction 8.

(ii) Spectrophotometric examination of fractions 4, 8 and 4*a* revealed that each exhibited an absorption band at 2600—2650 A. Attempts to precipitate the derivative from an aqueous solution of the polysaccharide material indicated that intact nucleic acid was not present but that the contaminant was probably a nucleoside. From the spectrographic determinations, it was calculated that the fractions 4, 8, and 4*a* would contain 3·9, 13·7, and 2·5% respectively of such a substance.

(iii) The nucleic acid derivative was not precipitated from an aqueous solution by either lanthanum acetate or dilute or glacial acetic acid.

Separation of the Nucleic Acid Impurities by Adsorption on an Ion-exchange Resin.—The lipoid-polysaccharide (fraction 8; $[\alpha]_D^{17°}$ + 28°; 50 c.c.; 2% solution) was twice passed slowly through a "Zeocarb" resin and then 10 times through a "Deacidite" resin. The removal of nucleic acid was followed by the Dische test which initially was strongly positive and finally was only weak.

The final traces of the nucleic acid were removed when the polysaccharide was precipitated with alcohol from the acidified (pH 2) final liquor. A small precipitate (8*a*; 0·05 g.) having a strong Dische test was first obtained. The acid alcoholic mother liquor was neutralised, and immediately a flocculent precipitate (8*b*; 0·7 g.) of polysaccharide, $[\alpha]_D^{16°}$ + 25°, was obtained.

Alkaline Hydrolysis of Polysaccharides, $[\alpha]_D^{17°}$ + 85° *and* $[\alpha]_D^{17°}$ + 25°.—A 0·5% solution of the somatic polysaccharide, in 2N-sodium hydroxide, was heated on a water-bath for 20 hours. No change in optical rotation ($[\alpha]_D^{17°}$ + 85°) could be detected, and the substance was recovered unchanged in good yield.

Similarly, no changes were detected when a 0·5% solution of lipoid polysaccharide in 2N-sodium hydroxide was treated in the same way.

Location of Polysaccharides, $[\alpha]_D^{17°}$ + 85° *and* $[\alpha]_D^{17°}$ + 25°.—(i) Dry defatted cells (12 g.) were shaken at room temperature for 12 hours with sand and water (40 c.c.). The cell debris was separated (centrifuge) and the supernatant liquor treated with N-sodium hydroxide (40 c.c.) at 100° for 20 hours. The solution was acidified (Congo-red) with acetic acid, filtered, and treated with alcohol (4 vols.). The resulting precipitate was taken up in water, dialysed for 24 hours, and reprecipitated (yield, 0·035 g.).

The product, which was evidently a somatic polysaccharide, had the following properties: iodine coloration, −ve; Dische test, −ve; Molisch test, +++ve; $[\alpha]_D^{17°}$ + 78° in N/2-sodium hydroxide.

(ii) 10 G. of ether-soluble lipoids separated from the organisms were saponified by being heated at

100° for 20 hours with sodium hydroxide (N; 50 c.c.). The liquor was filtered, acidified (Congo-red), and the resulting precipitate removed (centrifuge). To the supernatant liquid was added alcohol (5 vols.). The resulting precipitate was purified by dialysis in a cellophane membrane; it then had the following properties: iodine coloration, −ve; Dische test, ±ve; Molisch test, +++ve; $[\alpha]_D^{17°}$ + 27° in N/2-sodium hydroxide.

Serological Activity.—The polysaccharides, $[\alpha]_D^{17°}$ + 85° and $[\alpha]_D^{17°}$ + 25° (fractions 4*c* and 8), were submitted to the precipitin test with tuberculosis anti-sera. Both reacted in a dilution of 1 : 2,000,000.

TABLE III.

Properties of Polysaccharides, $[\alpha]_D^{18°}$ + 85° *and* $[\alpha]_D^{18°}$ + 25°.

	Somatic polysaccharide.	Lipoid-bound polysaccharide.
$[\alpha]_D^{18°}$	+85°	+25°
Ash, %	1·4	2·5
Inorganic phosphorus, %	0·6	0·5
Organic phosphorus, %	0·3	1·4
Nitrogen, %	0·73	1·7
Acid equivalent	2,300	1,470
Nucleic acid, %	2·5	13·7
Serological activity	1 : 2,000,000	1 : 2,000,000
Ehrlich test	±	+

These approximate very closely to the specific polysaccharides isolated by Heidelberger and Menzel (*J. Biol. Chem.*, 1937, **118**, 79).

Methylation of the Somatic Polysaccharide, $[\alpha]_D^{18°}$ + 85°.—The polysaccharide (25 g.) was methylated in 5 g. portions by sodium hydroxide and methyl sulphate, in the following manner: the material (5 g.) was dissolved in water (25 c.c.) and warmed to 35°. To this solution, 300 c.c. of 30% sodium hydroxide and 100 c.c. of methyl sulphate were added in aliquot tenth portions every 10 minutes; 30 c.c. of the sodium hydroxide being added first in order to maintain alkaline conditions. The reaction mixture, which was vigorously agitated during the addition, was stirred for a further 2 hours at 35° after all the reagents had been added.

The reaction liquid was then raised to 100° for 30 minutes and allowed to cool while still being stirred. The liquid was cooled in ice and acidified (Congo-red) with dilute sulphuric acid. The material which separated at this stage was carefully removed. The acidified liquor was dialysed (parchment) for 48 hours and concentrated to a small volume (30 c.c.). This solution containing some product, and the partially methylated material obtained on acidification, were mixed and remethylated.

After this second methylation almost all the product separated on acidification. A small amount of remaining material was extracted with chloroform from the solution.

After 5 methylations, the product was acetylated by treatment with methyl alcohol (40 c.c.) and acetic anhydride (10 c.c.) for 48 hours at room temperature. In this way any free amino-residues in the amino-sugar, the presence of which was indicated by a positive Ehrlich test in the original polysaccharide, would be acetylated at the nitrogen atom and would thus be in a suitable form for the hydrolysis of the methylated polysaccharide.

The methoxyl content was constant (39·9%) after 6 methylations. Yield 4·2 g. By repetition of this procedure, 20 g. of methylated polysaccharide were obtained from 24 g. of starting material. An attempt was made to increase the methoxyl content of the derivative by methylation with methyl iodide and silver oxide. After several such treatments, the derivative was recovered in good yield, with the methoxyl content unchanged. It was concluded, therefore, that a fully methylated product was obtained by the 6 initial methylations.

Fractionation of the Methylated Somatic Polysaccharide.—Since traces of deoxyribonucleic acid were present in the starting material it was necessary to ascertain whether or not these had been removed during methylation. The methylated derivative (5·4 g.), which was a brown viscid gum, was dissolved in dry chloroform (42 c.c.), and to the solution was added light petroleum (b. p. 60—80°) until a precipitate formed. This was removed and more petroleum added to the mother liquor until a second precipitate formed. In this way 4 fractions were separted, the fifth being obtained on evaporation of the final mother liquors. A typical fractionation is shown in Table IV.

TABLE IV.

No.	Petroleum added (c.c.).	Wt. (g.).	Ash, %.	OMe, %.	$[\alpha]_D$.*	Remarks.
M1	70	0·15	32·2	—	—	Inorganic impurity.
M2	100	2·35	Trace	39·5	+129°	Methylated polysaccharide.
M3	150	2·1	—	39·9	+125	—
M4	170	0·9	—	39·2	+122	—
M5	evaporation	0·5	—	43·1	+ 83	Not examined.

* In chloroform.

The fractions M2 and M3, consisting of essentially homogeneous methylated polysaccharide, were pale-yellow, glass-like solids, containing 0·3% of nitrogen and having no characteristic absorption band in the ultra-violet region. The material was phosphorus free.

Methanolysis.—A typical sample (0·44 g.) of the methylated polysaccharide ($[\alpha]_D^{20°}$ + 125° in chloroform) was boiled in methanolic hydrogen chloride (1%; 45 c.c.). The hydrolysis was followed polarimetrically and the optical rotation became constant ($[\alpha]_D^{20°}$ + 47°) after 5 hours; this value did not change when the concentration of the acid was increased to 5% and the heating resumed.

The methanolysis proceeded more rapidly when another sample of the methylated polysaccharide ($[\alpha]_D^{20°}$ + 125° in chloroform) was heated with 5% methanolic hydrogen chloride, and the rotation became constant ($[\alpha]_D^{20°}$ + 45°) after 3 hours.

In this way, 9 g. of the fractionated methylated polysaccharide were hydrolysed. The acid was neutralised (litmus) with silver carbonate and the silver residue carefully washed with methanol. More silver residues were removed from the methanolic solution of hydrolysis products by addition of a little light petroleum. The solution was then evaporated to dryness by aeration at 50°, giving a mixture of glycosides (8·8 g.).

Fractionation of the Mixed Glycosides.—The mixed glycosides (8 g.) were fractionally distilled in a Widmer flask in a high vacuum. The fractionation was controlled by noting frequently the change in the refractive index of the distillate. The rate of distillation was so adjusted that the process occupied about 24 hours. Nine fractions (I to IX) were thus collected (Table V). The still residue was re-hydrolysed with methanolic hydrogen chloride, and the further yield of mixed glycosides obtained was fractionally distilled in the manner described giving 5 fractions (X to XIV) (Table VI). The final still residue amounted to 0·9 g.

TABLE V.

No.	Vap. temp./ 0·02 mm.	Bath temp.	Wt. (g.).	$n_D^{17°}$.	OMe, %.	Remarks.
I	71—76°	106—116°	0·81	1·4471	51·5	Mobile colourless liquid.
II	76—78	117—122	0·87	1·4520	53·0	,, ,, ,,
III	78—81	122—125	1·02	1·4532	52·1	,, ,, ,,
IV	79—83	125—130	0·42	1·4550	50·1	Immobile colourless syrup.
V	83—84	133—138	0·32	1·4549	47·5	,, ,, ,,
VI	90	138—140	0·04	1·4569	48·3	,, ,, ,,
VII	94—97	150	0·72	1·4590	47·2	Viscid yellow syrup.
VIII	100	155—160	0·67	1·4630	47·8	,, ,, ,,
IX	>100	170—190	0·07	1·4640	44·4	,, ,, ,,

TABLE VI.

Re-hydrolysis of the still residue from first fractionation.

No.	Vap. temp./ 0·02 mm.	Bath temp.	Wt. (g.).	$n_D^{17°}$.	OMe, %.	Remarks.
X	68—72°	110—125°	0·40	1·4481	51·0	Colourless mobile liquid.
XI	79—82	125—145	0·30	1·4516	52·1	,, ,, ,,
XII	100—110	150—160	0·62	1·4620	45·2	Viscid colourless syrup.
XIII	120—125	160—200	0·38	1·4712	41·4	,, ,, ,,
XIV	120—	220—	0·11	1·4749	33·3	Viscid yellow syrup.
Still residue		—	0·9	—	—	—

Identification of 2 : 3 : 4-*trimethyl methyl*-L-*rhamnoside and* 3 : 5-*dimethyl methyl*-D-*arabinoside.* A specimen of the mixed fractions I and X (0·4 g.) was methylated with silver oxide and methyl iodide. The fully methylated glycosides so obtained afforded two fractions on fractional distillation. The first of these fractions (IA) distilled at 110°(bath temp.)/11 mm. and had $n_D^{21°}$ 1·4370 and OMe, 58·9%. The second fraction (IB) distilled at 90—93°/0·2 mm. and had $n_D^{21°}$ 1·4445 and OMe, 57%.

The fraction IA was readily hydrolysed by N/20-sulphuric acid ($[\alpha]_D$ + 31·5° ⟶ + 18°, 6 hours) which suggested the presence of a furanose structure. The free sugar so obtained did not form a crystalline anilide when boiled with alcoholic aniline. This free sugar (0·2 g.) was therefore oxidised with bromine (0·5 g.) in water (10 c.c.) at room temperature for 5 days. Excess of bromine was removed by aeration, and the solution was neutralised with silver carbonate. After filtration, hydrogen sulphide was passed in and the filtered solution evaporated to dryness. The syrupy acid produced (0·1 g.) was extracted from the residue with chloroform, and lactonised by being heated at 100° in a vacuum for 3 hours. This syrupy lactone, on treatment with dry saturated methanolic ammonia (20 c.c.) for 24 hours, yielded an amide which was recrystallised from acetone; m. p. 135—136° alone or in admixture with an authentic specimen of 2 : 3 : 5-trimethyl D-arabonamide {Found : $[\alpha]_D^{17°}$ − 14° in water, OMe; 43·9%. Cf. $[\alpha]_D$ − 14° in water; OMe, 44·9% (Haworth, Peat, and Whetstone, *J.*, 1938, 1975)}.

Fraction IB resisted hydrolysis with N/20-sulphuric acid but was hydrolysed with N-sulphuric acid ($[\alpha]_D^{19°}$ + 14° ⟶ + 9°; 4 hours). The free sugar derivative ($n_D^{17°}$ 1·4561; OMe, 45%) was converted into the anilide by being refluxed for 5 hours with the theoretical amount of aniline in dry ethanol. The syrup obtained after removal of the solvent was crystallised from light petroleum; m. p. 112° alone or in admixture with 2 : 3 : 4-trimethyl L-rhamnose anilide (Found : $[\alpha]_D^{19°}$ + 127°; OMe, 32·6%).

A sample of fractions I and X was hydrolysed first with N/20 then by N-sulphuric acids for 6 hours ($[\alpha]_D$ + 29° ⟶ ± 0°). A specimen of the free sugar derivative was converted into the anilide, which had m. p. 111—112° alone or in admixture with 2 : 3 : 4-trimethyl rhamnose anilide. A second anilide (m. p. 118°; $[\alpha]_D^{18°}$ − 28·8° in water after 2 hours) crystallised from the mother liquors on being kept; it was probably a dimethyl arabinose anilide, but was clearly different from 2 : 3-dimethyl arabinose anilide, m. p. 139° (Smith, *J.*, 1939, 753), and 2 : 4-dimethyl arabinose anilide, m. p. 142—143° (*idem*, *ibid.*, p. 744).

A further specimen of the free sugar derivative of I (0·3 g.) was oxidised with bromine (1 g.) at room temperature for 2 days. Excess of bromine was removed, and the lactone formed by distillation of the acid at 138°/0·05 mm. Treatment of the lactone with methanolic ammonia furnished an amide which on recrystallisation from acetone had m. p. 144° ($[\alpha]_D$ − 11° in water; OMe, 32·7%). This substance (10 mg.) was treated with sodium hypochlorite (5 drops) for 12 hours, and to it was then added saturated

semicarbazide hydrochloride and sodium acetate; a precipitate of hydrazodicarbonamide (m. p. 254°) was obtained. It is considered that this amide is 3 : 5-dimethyl D-arabonamide (cf. 3 : 5-dimethyl L-arabonamide, m. p. 145°; $[\alpha]_D$ + 10°; OMe, 31·9%; Cunneen, Thesis 1940, University of Birmingham; White, *J. Amer. Chem. Soc.*, 1946, **68**, 272).

Further quantities of 2 : 3 : 4-trimethyl methyl-L-rhamnoside and 3 : 5-dimethyl methyl-D-arabinoside were identified in fractions II, III, IV, and XI.

Identification of 3 : 4 : 6-*trimethyl methyl*-D-*mannoside.* A specimen (0·3 g.) of fractions VII, VIII, and XII was methylated with silver oxide and methyl iodide. The methylated product (0·3 g.; $n_D^{19°}$ 1·4450; b. p. 90—95°/0·02 mm.; OMe, 60·6%) was hydrolysed by being heated with N-sulphuric acid ($[\alpha]_D^{20°}$ + 47° ⟶ + 41° after 8 hours), and a specimen of the free sugar (0·1 g.; n_D 1·4600; OMe, 52·2%) converted into the anilide which readily crystallised from acetone–light petroleum; m. p. 140° alone or in admixture with 2 : 3 : 4 : 6-tetramethyl D-mannose anilide.

A specimen of the original mixed fractions VII, VIII, and XII (1 g.) was hydrolysed with N-sulphuric acid (25 c.c.) ($[\alpha]_D^{20°}$ + 53° ⟶ + 47° after 8 hours). The free sugar (0·85 g.; $n_D^{20°}$ 1·4778) was extracted with ether and crystallised from alcohol–ether. After recrystallisation, the substance, which strongly reduced Fehling's solution, had OMe, 40·6%, $[\alpha]_D^{20°}$ + 26° ⟶ + 6° in water (2 hours), and m. p. 100° alone or in admixture with 3 : 4 : 6-trimethyl D-mannopyranose (m. p. 102°; OMe, 41·8%; $[\alpha]_D^{18°}$ + 22° ⟶ + 8° in water; Haworth, Heath, and Peat, *J.*, 1941, 833).

The residue (0·2 g.) crystallised on standing at 0°, affording 3 : 4-dimethyl D-mannopyranose (see below).

Identification of 3 : 4-*dimethyl methyl*-D-*mannopyranoside.* A specimen (0·15 g.) of the mixture of fractions IX and XIII was methylated with silver oxide and methyl iodide. Hydrolysis of the product (0·12 g.; $n_D^{18°}$ 1·4556) with N-sulphuric acid (10 c.c.; $[\alpha]_D^{20°}$ + 27° ⟶ + 14°) afforded a free sugar derivative (0·08 g.; $n_D^{19°}$ 1·4670) which on treatment with ethanolic aniline gave a quantitative yield of 2 : 3 : 4 : 6-tetramethyl D-mannose anilide (m. p. 139° alone or in admixture with an authentic specimen).

A further specimen (0·3 g.) of fractions IX and XIII was hydrolysed with N-sulphuric acid (15 c.c.; $[\alpha]_D^{20°}$ + 46° ⟶ + 24° after 8 hours at 100°). The free sugar was extracted with ether. Evaporation of the ethereal extract gave a crystalline compound which, after recrystallisation from aqueous alcohol, had m. p. 113° alone or in admixture with 3 : 4-dimethyl D-mannopyranose monohydrate (Found : OMe, 27·5. Calc. : OMe, 27·48%; cf. Haworth, Hirst, and Isherwood, *J.*, 1937, 784).

Isolation of an amino-sugar derivative. On analysis it was found that the principal fractions obtained on distillation had the following nitrogen contents : mixed fractions I and X (0·27%); mixed fractions II, III, XI (0·33%); mixed fractions VII, VIII, XII (0·36%); mixed fractions IX, XIII (nil).

0·4 G. of each nitrogen-containing fraction was mixed and heated at 100° for 8 hours with sodium hydroxide (15 c.c.; 4N). The resulting solution was neutralised (phenolphthalein) with dilute sulphuric acid, extracted exhaustively with ether, and the extract then dried (Na_2SO_4) for 24 hours. After filtration, the dry ethereal solution was saturated with dry hydrogen chloride at 0°. A turbidity developed and a syrup separated. This was removed (0·03 g.; OMe, 32·4%) and washed with ether, then acetylated by being shaken for 12 hours with acetic anhydride (0·4 c.c.) and silver acetate (0·5 g.) in 10 c.c. of dry methanol. The filtered solution was evaporated to 2 c.c. and finally dried in a vacuum (at room temperature) over solid potassium hydroxide. A solid which was recrystallised from aqueous alcohol (m. p. 87°; 10 mg.) was obtained.

In view of the positive Ehrlich reaction of the original polysaccharide, the nitrogen contents, and the isolation of the above derivative (OMe, 32·4. Calc. for a dimethyl methylacetamidohexoside: OMe, 35·1%) it is suggested that this represents the amino-sugar constituent of the polysaccharide.

TABLE VII.

Assessment of the methylated sugar components of the methylated somatic polysaccharide.

Distillates, 6·35 g. Still residue, 0·88 g. (Ash, 13·4%).

Component.	Wt. (g).	% (by wt.).	*M*.	Mol. proportion.	% (mol.).	Molecular ratio.
3 : 5-Dimethyl methyl-D-arabinoside	1·40	22·0	192	7·29	29	5·05
2 : 3 : 4-Trimethyl methyl-L-rhamnoside	1·57	24·7	220	7·14	25·5	4·96
3 : 4 : 6-Trimethyl methyl-D-mannoside	1·70	26·8	236	7·20	25·6	4·99
3 : 4-Dimethyl methyl-D-mannoside	1·29	20·2	222	5·86	20·8	4·03
Dimethyl methylaminohexoside	0·38	5·9	263·5	1·44	5·1	1

Periodic Acid Oxidation (private communication from Dr. J. K. N. JONES).—51·54 Mg. of somatic polysaccharide were oxidised for 190 hours with sodium periodate (5 ml.; 0·274M), water (25 ml.), and potassium chloride (1 g.) with shaking. Excess of glycol was added at the completion of the oxidation, and the resulting solution titrated with barium hydroxide (6·77 ml.; 0·01N). (The initial substance was neutral.)

Thus 1 mol. of acid is given by 761 g. of polysaccharide. The unmethylated structure in (I) has *M*, 3051, *i.e.*, 3051 G. of polysaccharide give rise to 5 end-groups = 5 mols. of acid
610 g. of polysaccharide (theory) = 1 mol. of acid
But from experiment 761 g. of material = 1 mol. of acid
∴. No. of end groups (experimental) = 4

45·77 Mg. of the same polysaccharide were oxidised by the same method, and the uptake of IO_4^- was determined.

144 G. of polysaccharide took up 1 mol. of IO_4^-.

Structure (V) can take up 20 mols. of IO_4^- (assuming that the amino-sugar takes 1 mol.).

Thus, 153 g. of polysaccharide would take up 1 mol. of IO_4^- (theoretical value).

The authors thank Professor F. Smith for numerous suggestions, and express their gratitude for the help given by members of the staff of the Ministry of Agriculture and Fisheries Veterinary Laboratory at Weybridge. One of them (P. W. K.) thanks the Glaxo Laboratories, Ltd., for financial assistance which enabled him to take part in this and the following investigation. The serological tests were carried out by our colleague, Dr. E. Nassau.

THE UNIVERSITY, EDGBASTON, BIRMINGHAM, 15. [*Received, September 8th*, 1947.]

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241. *The Constitution of a Lipoid-bound Polysaccharide from* M. tuberculosis (*Human Strain*).

By SIR NORMAN HAWORTH, P. W. KENT, and M. STACEY.

The lipoid-bound specific polysaccharide fraction ($[\alpha]_D^{18°}$ + 25°) of *M. tuberculosis* was obtained from lipoid complexes extracted from the heat-killed organisms by organic bases.

The polysaccharide was converted into the methyl ether which on acid hydrolysis furnished 2 : 3 : 5-trimethyl methyl-D-arabofuranoside (12·8%), 3 : 5-dimethyl methyl-D-arabofuranoside (30·9%), 2 : 3 : 6-trimethyl methyl-D-galactopyranoside (33·8%), 3 : 4-dimethyl methyl-D-mannopyranoside (14·4%), and a dimethyl methyl-D-glucosaminide (8%).

The polysaccharide is shown to have a highly branched chain structure containing units of D-galactopyranose, D-mannopyranose, D-glucosamine, and D-arabofuranose, some of the last being present as end groups.

IN an investigation of complex substances of possible antigenic activity derived from *M. tuberculosis* (human strain), it was found that fractions could be separated from heat-killed cells by the action of basic organic substances (*e.g.*, urea, β-hydroxypropionamidine, etc.). Some of the fractions were highly serologically active when tested by the collodion particle agglutination test (Cannon and Marshall, *J. Immunol.*, 1940, **38**, 365; Riodoran, *Proc. Soc. Exp. Biol. Med.*, 1942, **49**, 622).

It was found that the most active fractions contained lipoids, deoxyribonucleic acid, and a serologically specific polysaccharide ($[\alpha]_D^{18°}$ + 25°), which had properties identical with the lipoid-bound polysaccharide isolated previously (Haworth, Kent, and Stacey, preceding paper) by alkaline extraction of *M. tuberculosis* cells.

The lipoid-bound polysaccharide obtained either by fractionation of the mixed polysaccharide of defatted cells or by alkaline treatment of the antigenic complex (see experimental section) was methylated with sodium hydroxide and methyl sulphate. After six treatments with the reagents the crude methylated derivative had a constant methoxyl content of *ca.* 42% and was in the form of a brown gum which was fractionated by precipitation from organic solvents.

This methylated derivative (20·5 g.) was hydrolysed by being refluxed with methanolic hydrogen chloride ($[\alpha]_D^{17°}$ + 31·5° ⟶ + 17°, 9 hours). The resulting mixture of glycosides was fractionated by distillation in a high vacuum. Five substances were identified :

(*a*) 2 : 3 : 5-Trimethyl methyl-D-arabinoside, identified by oxidation with bromine to the corresponding lactone and thence to 2 : 3 : 5-trimethyl D-arabonamide.

(*b*) A fraction (OMe, 48·5%) which underwent further methylation when treated with silver oxide and methyl iodide. Oxidation of the completely methylated product (OMe, 60%) afforded a lactone which was readily converted into 2 : 3 : 5-trimethyl D-arabonamide.

The initial fraction (*b*) was hydrolysed with N/20-sulphuric acid ($[\alpha]_D^{17°}$ − 6° ⟶ − 40°, 6 hours) which suggested the possible existence of a furanose structure. Treatment of a specimen of the free sugar derivative with alcoholic aniline yielded a crystalline anilide identical with the dimethyl pentose anilide from the methylated somatic polysaccharide (see previous paper).

The free sugar was oxidised with bromine, and the lactone so formed was converted into 3 : 5-dimethyl D-arabonamide.

(*c*) A fraction, having properties corresponding to those of a trimethyl methylhexoside, was methylated further, and hydrolysed to the free sugar which was then converted into 2 : 3 : 4 : 6-tetramethyl D-galactose anilide.

The original fraction (*c*) was hydrolysed directly to the free sugar which was oxidised to the acid. On being distilled in a high vacuum a crystalline 2 : 3 : 6-trimethyl D-galactonolactone was obtained (cf. Haworth, Raistrick, and Stacey, *Biochem. J.*, 1935, **29**, 2668).

(*d*) A sample of a fraction having OMe, 43·6% was methylated, and converted into 2 : 3 : 4 : 6-tetramethyl D-mannose anilide.

A further sample of this fraction (*d*) was hydrolysed. The free sugar crystallised on standing and was identified as 3 : 4-dimethyl D-mannopyranose.

(*e*) The amino-sugar constituent of the hydrolysed methylated polysaccharide was separated in the following manner. The acidic methanolic hydrolysate was diluted with water, the methanol removed by evaporation, and thereafter the mixed glycosidic constituents were obtained from the solution by continuous extraction with chloroform. The partially methylated amino-sugar hydrochloride remained in the aqueous liquid. The hydrochloride was isolated as a syrup. After acetylation of the amino-group and methylation of free hydroxyl groups, *N*-acetyl 3 : 4 : 6-trimethyl α-methyl-D-glucosaminide was obtained.

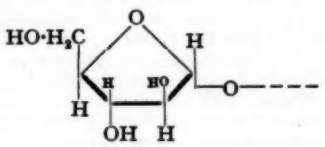
Examination of all the syrups obtained by distillation afforded only further amounts of these five components.

Discussion.

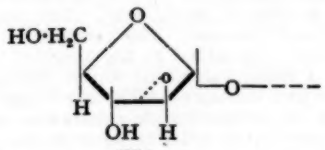
A quantitative assay of these constituents was made by reference to the refractive indices and methoxyl and nitrogen contents of the various glycosidic fractions. The following results were obtained: 2 : 3 : 5-trimethyl methyl-D-arabinoside (12·8%); 3 : 5-dimethyl methyl-D-arabinoside (30·9%); 2 : 3 : 6-trimethyl methyl-D-galactoside (33·8%); 3 : 4-dimethyl methyl-D-mannoside (14·4%); dimethyl methyl-D-glucosaminide (8%). This corresponded to a molecular ratio of 2 : 5 : 5 : 2 : 1 respectively.

The identification of 2 : 3 : 5-trimethyl methyl-D-arabinoside as a constituent of the methylated polysaccharide indicated that D-arabofuranose is combined glycosidically and is therefore an end group of the polysaccharide linked as in (I).

Since 3 : 5-dimethyl methyl-D-arabinoside was also present, it is clear that further D-arabofuranose residues are combined in the polysaccharide chain and are linked through the 1 and 2 positions as in (II).



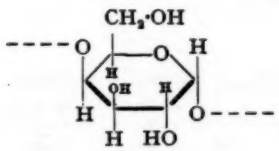
(I.)



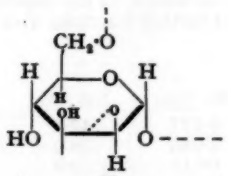
(II.)

The occurrence of 2 : 3 : 6-trimethyl methyl-D-galactoside establishes that D-galactose is present in the polysaccharide chain and is probably linked through positions 1 and 4 as in (III).

D-Mannopyranose is linked through the 1, 2, and 6 positions in the polysaccharide (IV), and reflects the degree of branching.



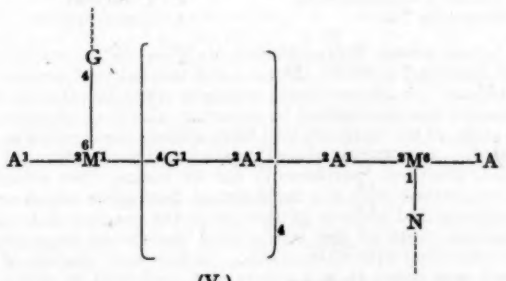
(III.)



(IV.)

The precise mode of linkage of the glucosamine residue which forms part of the chain has not yet been determined.

It is possible, in view of these results, to depict several possible configurations for this polysaccharide, one type of which is given in (V).



(V.)

A represents D-arabofuranose. M represents D-mannopyranose.
G represents D-galactopyranose. N represents D-glucosamine.

In this representation the linkages engaged glycosidically at C_1 are provisionally shown as being in the α-form in view of the change of rotation on hydrolysis of both the polysaccharide ($[\alpha]_D^{18°}$ + 28° ⟶ − 16°) and its methylated derivative ($[\alpha]_D^{18°}$ + 30° ⟶ − 15°) which are in the negative sense.

Work on determination of the molecular weight of this polysaccharide by osmotic pressure and oxidation methods is proceeding. The periodic oxidation method gives results which are less satisfactory than those for the somatic polysaccharide.

Experimental.

Isolation of the Complex.—Moist heat-killed cells were stirred with an equal weight of urea and warmed at 37° for 100 hours. The residual fluid was diluted with water (½ vol.) and centrifuged (2,500 r.p.m.) until no further solid material separated. To the supernatant liquid acidified (litmus) with dilute acetic acid, alcohol (5 vols.) was added, and the precipitated solid at once removed. This moist product was shaken with an equal volume of a 2% solution of sodium acetate and centrifuged. The residual solid was a complex consisting of free lipoid (soluble in organic solvents), a specific polysaccharide, $[\alpha]_D^{20°}$ + 28°, bound lipoids, and deoxyribonucleic acid (the last could be separated by extraction with dilute sodium hydroxide). The complex, as will be described elsewhere, gave, when combined with collodion, a strong agglutination reaction with sera from tuberculous patients.

Composition of the Serologically Active Urea Extract.—(i) *Determination of ether-soluble lipoid content.* The serologically active complex (4·4 g.) was extracted continuously for 30 hours with ether (1 l.) in a Soxhlet apparatus. Evaporation of the ethereal extract yielded a residue of lipoid material (0·628 g., 27%).

The initial fraction was acid-fast when stained by the carbolfuschin method. After the ether treatment, the defatted residue was non-acid fast though this property was retained by the extracted lipoids.

(ii) *Alkaline hydrolysis.* The ether-extracted fraction (2·5 g., 9·4%N) was heated at 100° for 30 hours with sodium hydroxide (40 c.c. of N). The unsaponified residue (1·1 g.) was removed and the supernatant liquid made acid (litmus). The precipitate, which consisted of peptide material, was collected, and alcohol (4 vols.) was added to the acidified mother liquor. The resulting precipitate was separated, dissolved in a small volume of water, dialysed through cellophane, and finally reprecipitated. The precipitate, which contained 4·7% of nitrogen, gave strong Molisch and Dische tests, and appeared to be composed of polysaccharide substances and deoxyribonucleic acid. A further fractionation was therefore carried out in order to separate the constituents of the mixture (see iii, below).

The alcoholic mother liquors, containing those fatty residues liberated during the saponification, were evaporated to dryness. A small amount (0·13 g.) of bound lipoid residues was obtained; the properties of this will be described elsewhere.

(iii) *Fractionation of the polysaccharide component.* The crude polysaccharide-containing precipitate isolated above was dissolved in water (50 c.c.), and alcohol was added dropwise to this aqueous solution, with stirring until a precipitate *F*1 was observed. This was collected and more alcohol was added, in the same manner as before, to the supernatant liquor, furnishing a precipitate *F*2. The procedure was continued until no further fractions were precipitated (Table I).

Table I.

No.	Wt. (g.).	Ash, %.	$[\alpha]_D^{18°}$.	Biuret.	Molisch.	Dische.	Bial.
*F*1	0·177	47·7	± 0°	—	++	+++	++
*F*2	0·065	26·0	+30	—	++	±	+
*F*3	0·119	19·9	+28	—	++	—	+
*F*4	0·06	1·8	+27	—	+	—	+
*F*5	0·03	2·1	+29	—	+	—	+
*F*6	0·18	35·0	+26	—	+	—	+

The crude polysaccharide mixture and the polysaccharide fraction *F*3, reacted in the precipitin test with sera from tuberculous patients in the following dilutions:

Crude polysaccharide	1 : 1,000,000
Fraction *F*3	1 : 2,000,000

Methylation of the Lipoid-bound Polysaccharide ($[\alpha]_D^{18°}$ + 25° in water).—The fractionated material (8 g.; $[\alpha]_D^{18°}$ + 25°) was dissolved in water (25 c.c.) and treated with sodium hydroxide (500 c.c. of 30%) and methyl sulphate (185 c.c.) in aliquot tenth portions every 10 minutes at 35° with vigorous stirring. Sufficient sodium hydroxide was first added to maintain alkaline conditions throughout. The mixture was stirred for 2 hours after all the reagents had been added, then cooled in ice and acidified (Congo-red) with dilute sulphuric acid. A quantity of methylated material separated at this stage. The aqueous solution was neutralised, dialysed (parchment) for 48 hours, then concentrated to a small volume (30 c.c.). This liquor was mixed with the methylated derivative which separated on acidification and treated with methyl sulphate and sodium hydroxide in the manner described.

After 3 such treatments most of the methylated derivative separated on acidification, and any remaining material was extracted with chloroform. In this way, dialysis of the aqueous liquors was not necessary. The product was dried in a vacuum and dissolved in dry pyridine (70 c.c.) and acetic anhydride (10 c.c.). After 48 hours at room temperature, the solvents were removed under reduced pressure (at room temperature) and the product again methylated. The methoxyl content was constant

after 6 methylations (yield 6 g.). By repetition of this procedure, 29 g. of methylated derivative were isolated.

Fractionation of the Methylated Polysaccharide.—The derivative (6·5 g.) was fractionated by graded precipitation from a chloroform solution (40 c.c.) with light petroleum. Five fractions were thus obtained and a further fraction was separated by evaporation of the mother liquors (Table II).

TABLE II.

No.	Petroleum added (c.c.).	Wt. (g.).	$[\alpha]_D^{20°}$.	OMe, %.	
1	—	0·9	—	—	inorganic residue
2	200	0·6	+ 9°	25·6	
3	98	2·9	+29	42·8	mainly methylated polysaccaride
4	102	0·5	+32	42·1	
5	135	0·9	+30	42·3	
6	evaporation	0·6	+25	31·0	

In this way, 21 g. of essentially homogeneous methylated polysaccharide were isolated, having N, 0·4; OMe, 42·3%; P, nil; and $[\alpha]_D^{19°}$ + 30°. Attempts to increase the methoxyl content of the substance by treatment with silver oxide and methyl iodide were unsuccessful, and it was therefore concluded that the product was already fully methylated.

Hydrolysis of the Methylated Polysaccharide.—A specimen (0·22 g.) was refluxed with methanolic hydrogen chloride (25 c.c.; 1%). The rate of hydrolysis was followed polarimetrically and was constant after 9 hours ($[\alpha]_D^{19°}$ + 31·5° ⟶ + 17°). When a further sample (0·10 g.) was hydrolysed with 5% methanolic hydrogen chloride, the reaction was completed in 1½ hours ($[\alpha]_D^{20°}$ + 34·0° ⟶ + 15·1°). There was no further change in optical rotation when the concentration of the acid was increased to 8%. The acid was neutralised with silver carbonate, and, after filtration, evaporation of the alcoholic solution gave a mixture of glycosides. From 20·5 g. of methylated polysaccharide, 19·5 g. of glycosidic hydrolysis products were obtained.

Fractional distillation of the products of hydrolysis. The mixture of glycosides (16·1 g.) was fractionally distilled from a Widmer flask in a high vacuum. Seven fractions were thus separated (Table III).

The still residue was rehydrolysed with methanolic hydrogen chloride (50 c.c.; 6%) for 4 hours, and the hydrolysis product again distilled, thus furnishing 6 further fractions (Table IV).

TABLE III.

No.	Wt. (g.).	Vap. temp.	Bath temp.	$n_D^{18°}$.	OMe, %.
1	1·31	66°	97—108°	1·4381	58·4
2	0·70	66— 84	109—129	1·4490	53·0
3	3·81	84— 90	129—132	1·4520	48·5
4	2·10	90— 95	132—154	1·4531	52·0
5	2·00	96—102	154—166	1·4520	50·1
6	1·13	102—117	175—189	1·4600	46·4
7	0·71	117—121	189—210	1·4705	43·6

TABLE IV.

Rehydrolysis of still residue.

No.	Wt. (g.).	Vap. temp.	Bath temp.	$n_D^{18°}$.	OMe, %.
8	0·04	55°	110°	1·4419	60·3
9	0·65	87— 89	122—125	1·4468	55·4
10	0·84	106—115	143—155	1·4533	53·6
11	0·43	104—106	155—163	1·4589	45·4
12	0·38	110—111	196—200	1·4660	45·6
13	0·85	120—130	220—240	1·4829	40·7

Still residue 0·9 g. Pressure 0·04 mm.

Identification of 2 : 3 : 5-*trimethyl methyl*-D-*arabinoside.* A sample of the fraction 1 (0·5 g.) was hydrolysed with N/20-sulphuric acid (25 c.c.) at 100° ($[\alpha]_D^{17°}$ + 63° ⟶ + 26°; 6 hours). The acid was neutralised (phenolphthalein) with sodium hydroxide, the solution evaporated to dryness, and the free sugar (0·45 g.; $n_D^{17°}$ 1·4470; OMe, 49·8%) extracted with ether. This sugar was oxidised by treatment with bromine (1 c.c.) at room temperature for 3 days. After aeration to remove unchanged bromine, the solution was neutralised with silver carbonate, filtered, and treated with hydrogen sulphide. After filtration, the liquid, which was non-reducing to Fehling's solution, was evaporated to dryness. The free acid so obtained was extracted with chloroform and lactonised by being heated at 100° for 3 hours in a vacuum.

The syrupy lactone was treated with dry methanolic ammonia (10 c.c.) for 12 hours at room temperature. On evaporation to dryness a syrup was obtained which crystallised rapidly on nucleation with 2 : 3 : 5-trimethyl D-arabonamide; m. p. 134—135° alone or in admixture with an authentic specimen (Found: OMe, 43·2%; $[\alpha]_D^{18°}$ − 25° in alcohol).

Identification of 3 : 5-*dimethyl methyl*-D-*arabinoside.* A specimen of fraction 3 (0·65 g.) was methylated by 3 treatments with methyl iodide and silver oxide. The fully methylated product (0·4 g.; b. p. 93—98°/0·2 mm.; $n_D^{19°}$ 1·4381; OMe, 60·8%) was hydrolysed with N/10-hydrobromic acid

($[\alpha]_D^{17°}$ − 3·5° ⟶ − 10°; 4 hours) and the free sugar oxidised with bromine. The lactone (0·2 g.; n_D 1·4590) was converted into the amide by treatment for 12 hours with saturated methanolic ammonia (5 c.c.). The product was crystallised from acetone; m. p. 135° alone or in admixture with 2 : 3 : 5-trimethyl D-arabonamide (OMe, 45·1%; $[\alpha]_D^{16°}$ − 13° in water).

A further specimen (1·1 g.) of the fraction 3 was hydrolysed by being heated with N/20-sulphuric acid (20 c.c.) at 100° for 9 hours ($[\alpha]_D^{18°}$ − 6·1° ⟶ − 40·0°). Some of the free sugar (0·08 g.; $n_D^{16°}$ 1·4712; OMe, 35·3%) was refluxed with alcoholic aniline (0·04 g. in 2 c.c.) for 6 hours. The anilide was crystallised from acetone–light petroleum; m. p. 118° (Found : OMe, 24·0. Calc. for a dimethyl pentose anilide : OMe, 23·1%).

The free sugar (0·6 g.) was oxidised with bromine (1·5 c.c.) for 4 days at room temperature. The acid, isolated in the usual way, was distilled [0·3 g.; b. p. 176° (bath temp.)/1 mm.; $n_D^{18°}$ 1·4521; OMe, 37·1%] and this product—a lactone—converted into the amide with methanolic ammonia and crystallised from acetone; m. p. 144°; OMe, 33%; $[\alpha]_D^{17°}$ − 10° in water [cf. m. p. 145°; $[\alpha]_D^{17}$ + 10° for 3 : 5-dimethyl L-arabonamide (Cunneen, Ph.D.Thesis, Birmingham University, 1940; White, *J. Amer. Chem. Soc.*, 1946, **68**, 272)].

Identification of 2 : 3 : 6-*trimethyl methyl*-D-*galactoside.* A sample of the mixed fractions 4, 5, 10 (0·5 g.) was methylated with silver oxide and methyl iodide. The product (0·4 g.; $n_D^{18°}$ 1·4420; OMe, 61·2%) was hydrolysed with N-sulphuric acid (10 c.c.) at 100° for 7 hours ($[\alpha]_D^{20°}$ + 0·6° ⟶ − 2·5°). The free sugar (0·2 g.; $n_D^{19°}$ 1·4560; OMe, 50·1%) was refluxed with alcoholic aniline (0·1 g. in 4 c.c.) for 6 hours. The anilide was crystallised from acetone–light petroleum; m. p. 186° alone or in admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl D-galactose anilide.

A further specimen of the mixed fraction (0·6 g.) was hydrolysed by being heated with N-sulphuric acid (20 c.c.) at 100° for 8 hours ($[\alpha]_D^{18°}$ − 4·0° ⟶ − 9·0°). The free sugar (0·3 g.; $n_D^{18°}$ 1·4620) was oxidised with bromine (1 g.) at room temperature for 5 days. On distillation of the acid so formed (b. p. 158°/0·02 mm.) a lactone was obtained which crystallised on being nucleated with 2 : 3 : 6-trimethyl D-galactonolactone. The material was recrystallised from ether–ethanol and had m. p. 96° alone or in admixture with 2 : 3 : 6-trimethyl D-galactonolactone (Found : OMe, 42·7. Calc. for $C_9H_{16}O_6$: OMe, 42·3%).

Identification of 3 : 4-*dimethyl methyl*-D-*mannoside.* A specimen (0·5 g.) of the mixed fractions 6, 11, 12 was methylated by 3 treatments with methyl iodide and silver oxide. The fully methylated product (0·45 g.; $n_D^{16°}$ 1·4460; OMe, 61·4%) was hydrolysed with N-sulphuric acid (10 c.c.) in 5 hours ($[\alpha]_D^{17°}$ + 6° ⟶ ± 0°). The free sugar (0·2 g.; $n_D^{17°}$ 1·4579; OMe, 52·3%) was converted into the anilide by being boiled with aniline (0·18 g.) in dry ethanol (6 c.c.) for 6 hours. Evaporation of the solvents yielded an anilide which was recrystallised from acetone–light petroleum; m. p. 138° alone or in admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl D-mannose anilide.

A specimen (0·5 g.) of the mixed fraction was hydrolysed ($[\alpha]_D$ + 13° ⟶ + 8°; 9 hours) with N-sulphuric acid (20 c.c.). The free sugar which crystallised on standing at 0° from ether–ethanol, had m. p. 108° alone or in admixture with 3 : 4-dimethyl D-mannose (Found : OMe, 29·9. Calc. for $C_8H_{16}O_6$: OMe, 29·8%).

Investigation of the Amino-sugar Constituent.—Methylated lipoid-bound polysaccharide [7 g.; OMe, 42·2%; $[\alpha]_D^{18°}$ + 30° in chloroform (*c*, 0·4)] was hydrolysed by being boiled with methanolic hydrogen chloride (10% : 100 c.c.) for 36 hours. Water (100 c.c.) was added to the hydrolysate, and the methanol removed under reduced pressure. The acid aqueous solution so obtained was extracted continuously with chloroform for 80 hours. The aqueous layer was separated and concentrated in presence of solid sodium hydroxide. A syrup (0·3 g.; OMe, 35·8%) was obtained, which, after removal of a small amount of inorganic impurity, was acetylated by being shaken with acetic anhydride (0·5 g.) and dry silver acetate (0·6 g.) in dry methanol (20 c.c.). The mixture was shaken overnight at room temperature, and after filtration the solution was evaporated to dryness. The resulting syrup (0·15 g.) was methylated 5 times using silver oxide and methyl iodide. A crystalline solid was obtained having m. p. 150° alone or in admixture with *N*-acetyl 3 : 4 : 6-trimethyl α-methyl-D-glucosaminide (Found: OMe, 44·9. Calc. for $C_{12}H_{23}O_6N$: OMe, 44·8%) (cf. Cutler, Haworth, and Peat, *J.*, 1937, 1979).

Assessment of the Methylated Sugar Components of the Methylated Polysaccharide.

Distillate weight 14·47 g. Still residue 0·9 g.

Component.	Wt. (g.).	% (by wt.).	*M.*	Mol. proportion.	% (mol.).	Molecular ratio.
2 : 3 : 5-Trimethyl methyl-D-arabinoside	1·86	12·83	206	9·03	13·5	2·05
3 : 5-Dimethyl methyl-D-arabinoside	4·48	30·95	192	23·33	34·9	5·3
2 : 3 : 6-Trimethyl methyl-D-galactoside	4·89	33·78	236	20·72	31·0	4·7
3 : 4-Dimethyl methyl-D-mannoside	2·08	14·37	222	9·36	14·0	2·1
Dimethyl methylglucosaminide	1·16	8·02	263·5	4·40	6·6	1·0

The serological tests were carried out by our colleague, Dr. E. Nassau.

THE UNIVERSITY, EDGBASTON, BIRMINGHAM, 15. [*Received, September 8th*, 1947.]

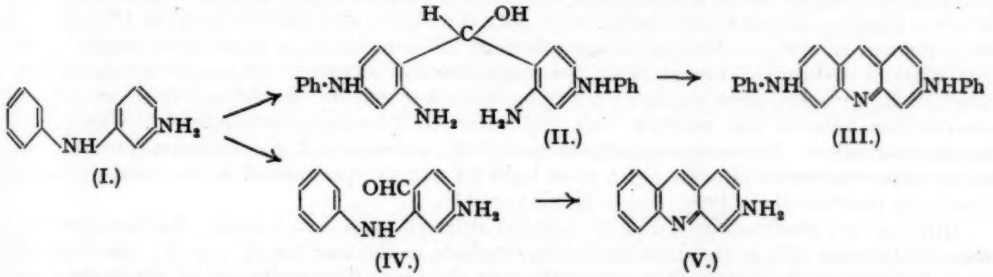
242. *Acridine Syntheses and Reactions. Part IV. A New Synthesis of Aminoacridines from Formic Acid and Diarylamines.*

By Adrien Albert.

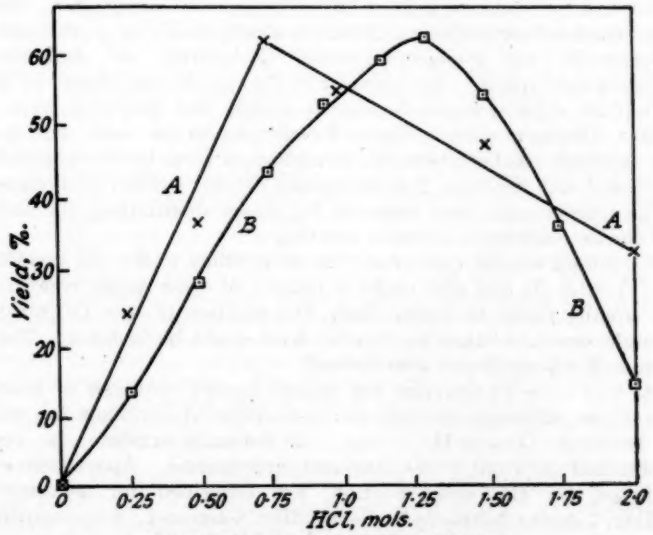
The reaction between formic acid and *N*-aryl-substituted *m*-diamines gives not only symmetrically-substituted diaminoacridines, according to the reaction described in Parts I—III (Albert, *J.*, 1941, 121, 484; 1947, 244) but also monoaminoacridines. The latter arise from a new reaction, which has a formal similarity to the Bernthsen synthesis and whose mechanism is discussed.

Experimental conditions can be regulated so that the new reaction preponderates, thus providing a useful preparative method for 2-aminoacridine and its derivatives.

It was shown (Part III) that derivatives of *m*-phenylenediamine, when heated with formic acid and hydrogen chloride, gave the corresponding derivatives of 2 : 8-diaminoacridine. For instance, *NN*-dimethyl-*m*-phenylenediamine gave 2 : 8-bisdimethylaminoacridine, and *N*-phenyl-*m*-phenylenediamine (3-aminodiphenylamine, I) gave 2 : 8-bisphenylaminoacridine (III) in 40% yield accompanied by a by-product, 2-aminoacridine (V).



By reducing the proportion of mineral acid, it has been found that progressively less (III) and more (V) were formed and a maximal yield of 2-aminoacridine (60%) was obtained when



Catalytic effect of hydrogen chloride.

(A) 2-*Aminoacridine from* 3-*aminodiphenylamine* (1 *mol.*) *and formic acid* (1 *mol.*).
(B) 2 : 7-*Diaminoacridine from* 3 : 4′-*diaminodiphenylamine* (1 *mol.*) *and formic acid* (1·25 *mols.*).

0·75 mol. of hydrogen chloride per mol. of amine was used (cf. Fig.). Under these conditions the yield of (III) was only 10%, and when still less mineral acid was used both reactions (I ⟶ V) and (I ⟶ II ⟶ III) were progressively suppressed. Finally, in the complete absence of hydrogen chloride, no more than a trace of acridines was produced even with a five-fold excess of formic acid.

It is evident that there are two competitive reactions. Both are catalysed by hydrogen ions, but (I ⟶ III) is favoured by a hydrogen-ion concentration which is above the optimum for (I ⟶ V). It is relevant that no less than 1·15 mols. of hydrogen chloride per mol. of *m*-phenylenediamine were required for maximal conversion of the latter into proflavine, a reaction which is the prototype of (I ⟶ III).

It was thought that reaction (I ⟶ V) was an example of the Bernthsen reaction, *i.e.*, the conversion of diphenylamines into acridines on heating with organic acids, preferably in the presence of zinc chloride, at 220—270° for 10 to 48 hours; for example, diphenylamine and acetic (or benzoic) acid give 5-methyl- (or 5-phenyl-) acridine in 50% yields (Bernthsen, *Annalen*, 1884, **224**, 3). The Bernthsen reaction, however, gives only a 7·5% yield with formic acid (Bernthsen, *loc. cit.*), whereas the new reaction gave no acridines with acetic or benzoic acids, even after heating for two hours at 185°, but gave a good yield with formic acid even after only one hour at 155°. Again, zinc chloride is prejudicial to the new reaction. These data suggest that this reaction may have a different mechanism from the Bernthsen reaction.

It is often assumed that the Bernthsen reaction proceeds through acylation of the secondary amino-group in diphenylamines. The new reaction, however, cannot proceed *via* 3-amino-*N*-formyldiphenylamine because this compound did not produce any 2-aminoacridine below 150° (whereas 3-aminodiphenylamine began to produce it at 120°), and the final yield at 175° was low (50% instead of 60%). This retarding effect of *N*-formylation is even more marked with 3 : 3′-diamino-*N*-formyldiphenylamine (see experimental section). Although *3-formamidodiphenylamine* gave the same yields as (I) under both wet and dry conditions, it is not a likely intermediate because the reaction was applicable to 3-hydroxydiphenylamine which gave 2-hydroxyacridine. Likewise *3-dimethylaminodiphenylamine* gave *2-dimethylaminoacridine*. In neither case was excess of acidity (up to at least 1·5 equivs.) prejudicial to the yields, possibly because no reaction of the type (I ⟶ III) is possible.

Although no intermediate could be isolated from the reaction mixture, 3-aminodiphenylamine-6-aldehyde (IV) is the most likely intermediate in the reaction (I ⟶ V), the aldehyde group entering at the point of greatest electron density. The synthesis of aldehydes from formic acid and amines may have a precedent in that *p*-dimethylaminobenzaldehyde is almost certainly an intermediate in the formation of 4 : 4′-bisdimethylaminobenzhydrol from dimethylaniline, formic acid, and hydrochloric acid at 120° (Votoček and Kraus, *Ber.*, 1909, **42**, 1604).

Attempts to synthesise 3-aminodiphenylamine-6-aldehyde (IV), *e.g.*, through *3-nitrodiphenylamine-6-carboxyhydrazide*, its p-*toluenesulphonyl* derivative, or *3-aminodiphenylamine-6-carboxyhydrazide*, gave only resins. An estimate of the ease of ring closure of (IV) was obtained by the examination of *diphenylamine-2-aldehyde* which was prepared from ethyl diphenylamine-2-carboxylate through *diphenylamine-2-carboxyhydrazide* and its p-*toluenesulphonyl* derivative. This aldehyde has been obtained in an impure form by Mayer and Stein (*Ber.*, 1917, **50**, 1306) who refluxed iodobenzene, 2-aminobenzaldehyde, sodium carbonate, and copper in nitrobenzene. The nitrobenzene was removed by steam distillation, the authors apparently not realising that the new aldehyde is steam-volatile.

This aldehyde cyclised almost quantitatively to acridine under the conditions which were used to produce (V) from (I) and also under a variety of other acidic conditions. If the ring closure of (IV) is equally facile, as seems likely, the reaction (I ⟶ IV) must be the limiting factor and it is understandable that no intermediate could be isolated. The ring closure of 2-nitrodiphenylamine-2′-aldehyde was also studied.

The new reaction (I ⟶ V) provides the easiest known synthesis of 2-aminoacridine and many of its derivatives, although symmetrically substituted acridines are more conveniently made by the reaction (I ⟶ III); *e.g.*, 2 : 8-diaminoacridine is best made from *m*-phenylenediamine and not from 3 : 3′-diaminodiphenylamine. Apart from examples already mentioned, syntheses, by the new reaction, are described for *2-dimethylaminoacridine*, 2 : 7-diaminoacridine, *7-amino-2-dimethylaminoacridine*, *8-amino-1 : 2-benzacridine*, and 8-amino-3 : 4-benzacridine. The diamines require 1·3 moles of hydrochloric acid per mole for optimal yields (cf. Fig.).

The reaction was not successful when a strong electron-repelling group was absent from the 3- position (*e.g.*, diphenylamine, 4-aminodiphenylamine, 3-nitrodiphenylamine). 3 : 2′-Diaminodiphenylamine gave only a benziminazole.

No 4-aminoacridine was obtained as a by-product in reaction (I ⟶ V), and 3-amino-6-methyldiphenylamine (which could not have given a derivative of 2-aminoacridine) did not yield any *4-amino-1-methylacridine*, which was prepared by an independent route for comparison. Hence the substances formed by the new reaction have all been formulated as

derivatives of 2-(and not 4-)aminoacridine. Confirmation of this orientation is derived from the relatively high basic strengths (forthcoming publication), and yellow (as distinct from violet) colours of the ions.

Experimental.*

Acridine Derivatives.

2-Aminoacridine (V).—3-Aminodiphenylamine (3·7 g.; 0·02 mole), glycerol (15 g.), formic acid (0·92 g. anhydrous; 0·02 mole), and hydrogen chloride (0·55 g.; 0·015 mole as 1·55 ml. of standardised acid) were raised to 155° during ½ hour, kept at 155° for ½ hour, then heated at 175° for ½ hour. When the dark red mass was cool, concentrated hydrochloric acid (0·5 ml.) and then water (40 ml.) were stirred in. The mixture was brought to the boil, hydrated sodium acetate (1 g.) was added, and the less basic by-products, including (III), were filtered off. 2-Aminoacridine was precipitated from the boiling filtrate with sodium hydroxide and recrystallised as the hydrochloride from 20 parts of 0·5N-hydrochloric acid. The base was again precipitated and recrystallised from alcohol. Yield 60%, m. p. 216°. Identity was confirmed by comparison (including mixed m. p.) with a specimen prepared by reducing 2-nitroacridone.

These conditions were optimal, even on the 100 g. scale. The yield was not improved by doubling the amount of formic acid or by longer heating at 175°, and was reduced to 40% by omission of the ½ hour's heating at 175°, to 50% by the use of substantially anhydrous conditions, and to 50% by the addition of 0·02 mole of zinc chloride. When hydrochloric acid was omitted, 3-formamidodiphenylamine, m. p. 125°, was obtained in 75% yield, the rest of the 3-aminodiphenylamine being unchanged.

The absence of 4-aminoacridine from all mother-liquors was established by concentration and precipitation with sodium hydroxide. The precipitate was washed and dissolved in 0·1N-acetic acid. The tip of a clamped strip of filter paper was then inserted. After 15 minutes, no violet band had appeared at the top of the wet area. This test will detect 1 part of the 4-isomeride in 200 parts of the 2-isomeride at a dilution of 1 in 1000.

When 3-acetamidodiphenylamine replaced 3-aminodiphenylamine in the above condensation, hydrolysis preceded condensation, as 2-aminoacridine (32% yield), but no 2-acetamidoacridine, was isolated.

2-Hydroxyacridine.—3-Hydroxydiphenylamine was condensed as above. The melt was boiled with water (20 ml.) and filtered hot. The extract and washings were precipitated at 90° with ammonia and filtered. The cake was washed with boiling water, and dissolved in hydrochloric acid. The solution was refrigerated, and the sparingly soluble hydrochloride collected, dissolved in boiling water, and precipitated with ammonia. The free base was dissolved in boiling alcohol (200 ml.) and concentrated until crystals began to separate. On cooling, orange crystals of 2-hydroxyacridine were obtained in 35% yield, m. p. 283° (sealed), undepressed by a specimen, m. p. 285°, synthesised by the method of Albert and Ritchie (*J.*, 1943, 458).

2-Dimethylaminoacridine.—3-Dimethylaminodiphenylamine was condensed and worked up, exactly as 3-aminodiphenylamine. The filtrate from the sodium acetate treatment was precipitated with sodium hydroxide and boiled until the oily base turned to a solid which was recrystallised from a little alcohol and then from benzene–light petroleum. *2-Dimethylaminoacridine* (60% yield) formed orange crystals, m. p. 183°, sparingly soluble in water, very soluble in alcohol with a yellow colour and strong green fluorescence (Found: C, 81·0; H, 6·3; N, 12·6. $C_{15}H_{14}N_2$ requires C, 81·0; H, 6·35; N, 12·6%).

The scarlet sulphate is very soluble in water, and the scarlet hydrochloride is not salted out by hydrochloric acid. The orange aqueous solutions are fluorescent (orange) only when dilute.

2 : 8-Diaminoacridine.—3 : 3′-Diaminodiphenylamine (Albert and Linnell, *J.*, 1936, 89) was condensed in the same way as its isomeride. The melt was boiled with excess of N-sodium hydroxide. The base was filtered off and extracted with N-acetic acid. The 2 : 8-diaminoacridine was precipitated from the filtrate and recrystallised from 55 parts of alcohol, giving a 55% yield of yellow crystals, m. p. (sealed) 276°, which did not depress the m. p. of a specimen prepared as in Part I. When the diaminodiphenylamine was replaced by 3 : 3′-diamino-*N*-formyldiphenylamine, the yield fell to 30% (cf. Part II).

2 : 7-Diaminoacridine.—3 : 4′-Diaminodiphenylamine (4·0 g.; 0·02 mole) was condensed in the same way as 3-aminodiphenylamine except that 0·026 mole (1·3 equivs.) of hydrochloric acid was used. The melt was digested at 90—100° for 5 minutes with sulphuric acid (8 ml. of 30% w/v), diluted to 80 ml. with water, and kept on ice for an hour. The acid sulphate of 2 : 7-diaminoacridine was filtered off, washed with water (10 ml.), then boiled with N-sodium hydroxide (25 ml.). The crystalline base was triturated with pyridine trihydrate (b. p. 93°; 10 ml.), filtered, and the residue recrystallised from about 100 parts of this solvent, giving 2 : 7-diaminoacridine in 60% yield, m. p. (sealed) 350° (long-stem), 355° (short-stem), approx. 365° (corr.). The results of varying the mineral acidity are shown in the Figure.

The base is soluble *ca.* 1 in 3,000 in boiling water, moderately soluble in nitrobenzene but without temperature gradient, and can be recrystallised from aniline, or from carbitol neutralised with morpholine. The monohydrochloride was formed by mixing the base (2·09 g.) with boiling 0·5N-hydrochloric acid (20 ml.), cooling to 50°, adding boiling acetone (40 ml.), and chilling (80% recovery). It is soluble in *ca.* 200 parts of cold water but is much more soluble (*e.g.*, 1 in 10) if a trace of hydrochloric acid is added. The acid sulphate, $C_{13}H_{11}N_3,H_2SO_4$, is soluble in 600 parts of boiling and 2,000 parts of cold water and is less soluble in the presence of excess of sulphuric acid.

7-Amino-2-dimethylaminoacridine.—4-Amino-3′-dimethylaminodiphenylamine was condensed exactly as 3 : 4′-diaminodiphenylamine. The melt was poured directly into sodium hydroxide solution and the oily base was collected and extracted with N-acetic acid, which left a tar. The extract was poured into

* Most of this work was carried out in 1941 but publication was delayed by a National Security Order, now revoked. Accordingly the data were filed at the Patent Office by the University of Sydney and the author. Australian and British Serial Nos. are respectively 115,480 (1941) and 572,766 (1945).

boiling N-sodium hydroxide and the base filtered off. A 1 in 5, hot solution of the neutral hydrochloride was then made and precipitated with an equal volume of acetone. The regenerated base was recrystallised from 7 parts of pyridine trihydrate and from 30 parts of 25% alcohol. *7-Amino-2-dimethylaminoacridine* (70% yield) formed orange needles, m. p. (sealed) 242° (249° corr.), very soluble in alcohol and methanol, less in acetone, slightly soluble in toluene and water, in all cases without temperature gradients. The orange solution in alcohol has a green fluorescence (intense yellow by ultra-violet light) (Found: C, 76·4; H, 6·3; N, 17·4. $C_{15}H_{15}N_3$ requires C, 75·9; H, 6·4; N, 17·6%).

The monohydrochloride gives intense bluish-red solutions with orange fluorescence, best seen under ultra-violet light. The dihydrochloride gives an orange solution with yellow fluorescence. Diazotisation is readily effected.

4-Amino-1-methylacridine.—4-Amino-1-methylacridan (Clemo, Perkin, and Robinson, *J.*, 1924, 1774) was suspended in boiling 50% alcoholic sodium hydroxide solution (N) and gently aerated for an hour. The oxidation was quantitative, although the solid acridan is not affected by long exposure at 20°. *4-Amino-1-methylacridine* forms scarlet solvated needles, from dilute alcohol or benzene, which fall to a scarlet powder at 120°; m. p. 128—129°. Unlike 4-aminoacridine, it does not turn yellow on hydration. The orange solution in alcohol has a slight orange fluorescence (Found: C, 80·8; H, 5·85; N, 13·5. $C_{14}H_{12}N_2$ requires C, 80·7; H, 5·8; N, 13·5%). The acetate and monohydrochloride are violet and their solutions do not fluoresce; the latter is precipitated from the solution of the base in 0·1N-hydrochloric acid by half saturation with sodium chloride. The dihydrochloride is formed in 2N-hydrochloric acid and is yellow. The yellow diazonium solution couples with β-naphthol (scarlet).

8-Amino-1:2-benzacridine.—α-Naphthyl-*m*-phenylenediamine hydrochloride (2·7 g.), formic acid (0·46 g.), water (0·8 ml.), and glycerol (9·4 g.) were condensed as before. The melt was repeatedly extracted with boiling water. The filtrate was basified and the precipitate extracted with N-acetic acid. The extract was treated with sodium acetate crystals (0·5 g.), filtered from weak bases, and precipitated with sodium hydroxide. The solid was dissolved in 50 parts of boiling alcohol, filtered, and concentrated to 10 parts. Recrystallisation from toluene gave *8-amino-1:2-benzacridine* (40% yield) as bright yellow crystals with a bronze reflex when large; m. p. (sealed) 200°. The alcoholic solution has a brilliant green fluorescence (Found: C, 83·4; H, 4·9; N, 11·45. $C_{17}H_{12}N_2$ requires C, 83·6; H, 5·0; N, 11·5%).

When 0·75 or 1·25, instead of 1·0 equiv. of hydrochloric acid was used, the yield fell to 30%.

The red hydrochloride is readily salted out by chloride ions. Its orange solution in water develops a green fluorescence on dilution, gives no colour change on diazotisation, but couples with β-naphthol (deep red).

Attempted synthesis of 8-amino-1:2-benzacridine from α-naphthol, 2:4-dinitrobenzyl chloride, and stannous chloride by the general method of Baezner and Gueorguieff for the β-isomeride (*Ber.*, 1906, **37**, 3082) gave a yield of only 1%.

8-Amino-3:4-benzacridine.—This was prepared similarly from β-naphthyl-*m*-phenylenediamine in 45% yield. After repeated recrystallisation from dilute alcohol and toluene the m. p. (sealed) remained at 264—265°; which was also the highest m. p. obtainable from material synthesised, in 10% yield, by the method of Baezner and Gueorguieff (*loc. cit.*), and a mixed m. p. showed no depression. It would seem that the m. p. (270°) quoted by these authors had been corrected.

On acetylation with acetic anhydride, specimens obtained by either method gave 8-acetamido-3:4-benzacridine quantitatively. The m. p. and mixed m. p.s of the crude products were 269°, and repeated recrystallisation from dilute alcohol did not change this figure. The above authors give m. p. 267° from nitrobenzene, but the base tends to decompose in this solvent.

The solubilities, colours, and fluorescences of base and acetyl derivative were as described by these authors.

Ring Closure of Diphenylamine-2-aldehyde.—(*a*) The aldehyde (0·55 g.), glycerol (2·0 g.), and standardised hydrochloric acid (0·26 ml. = 0·10 g. HCl = 1 equiv.), heated at 155° for 5 minutes, gave 95% of acridine, m. p. 110° (highest published m. p. is 111°). (*b*) Heating at 130° for 30 minutes gave 88%. (*c*) Heating at 130° for 5 minutes gave 26% of acridine and 67% of unchanged aldehyde. (*d*) The aldehyde (0·3 g.) in glacial acetic acid (1 ml.), heated with sulphuric acid (0·15 ml.; 4 equivs.) at 100° for 5 minutes, gave a 93% yield, m. p. 110° (for this cyclising method cf. Jensen and Rethwisch, *J. Amer. Chem. Soc.*, 1928, **50**, 1144). (*e*) The aldehyde (0·3 g.), heated with sulphuric acid (1·2 ml.) at 100° for ½ hour, gave a 98% yield of acridine, m. p. 110° (cf. Mayer and Stein, *loc. cit.*).

Ring Closure of 2-Nitrodiphenylamine-2′-aldehyde.—This substance (Albert and Ritchie, *J.*, 1943, 458) was investigated similarly. Method (*a*) gave a 36% yield of 1-nitroacridine, m. p. *ca.* 157°; (*d*) gave a 50% yield, m. p. 165°; and (*e*) gave a 98% yield, m. p. 165° (the m. p. of pure 1-nitroacridine is 167°). In each case the product was diluted with hot 0·5N-hydrochloric acid and filtered from unchanged aldehyde before precipitation of the base.

Diphenylamine Derivatives.

3-Aminodiphenylamine.—3-Nitrodiphenylamine (50 g.), Raney nickel (9 g.), and alcohol (250 ml.) were shaken with hydrogen at atmospheric temperature and pressure until the absorption was theoretical (2 hours). The catalyst was filtered off and was active through 3 more runs. The alcohol was recovered from the filtrate and the solid was distilled, giving 3-aminodiphenylamine, b. p. 190°/2 mm., m. p. 75°, in 90% yield. This substance was often obtained as a gum which turned to white crystals if stored at 50°. This amine (7·4 g.) and formic acid (2·6 ml.; 50% excess) were heated at 97° for an hour, cooled, diluted with water, and filtered. The damp precipitate was recrystallised from a little alcohol until the m. p. became constant, then dried in a vacuum, giving white crystals of *3-formamidodiphenylamine* in 75% yield, m. p. 135—136°, moderately soluble in benzene with a temperature gradient (Found: C, 73·1; H, 5·6. $C_{13}H_{12}ON_2$ requires C, 73·6; H, 5·7%). Unlike the isomeric 3-amino-*N*-formyldiphenylamine, m. p. 130—131° (Part II), it is insoluble in dilute hydrochloric acid and does not diazotise. A mixture of the isomerides began to melt at 100°.

3-Acetamidodiphenylamine.—3-Aminodiphenylamine (3·7 g.), acetic anhydride (3 ml.), and pyridine

(5 ml.) were mixed at − 5°. The mixture was left overnight at 20° and then poured into water (80 ml.). The precipitate was recrystallised from alcohol until the m. p. became constant, giving white crystals of *3-acetamidodiphenylamine*, m. p. 131° (95% yield), only moderately soluble in benzene and light petroleum (Found: C, 73·9; H, 6·3. $C_{14}H_{14}ON_2$ requires C, 74·3; H, 6·2%). The cold alcoholic solution gave a green colour with ferric chloride, in contrast to 3-amino-*N*-acetyldiphenylamine.

3-Aminodiphenylamine (7·6 g.) and acetic anhydride (15 g.) were heated for 15 minutes at 100°, cooled, and treated with benzene–light petroleum (2 : 1). The lower layer, when stirred with ether, gave a white solid (4·3 g.; m. p. 149—150°). The m. p. was not changed by recrystallisation from 10% alcohol or from water. The residues were taken to dryness and re-acetylated as above, giving altogether a 70% yield of *3-acetamido-N-acetyldiphenylamine*, soluble in 200 parts of boiling water, readily soluble in alcohol, less soluble in cold benzene, and almost insoluble in light petroleum (Found: N, 10·50. $C_{16}H_{16}O_2N_2$ requires N, 10·45%). Cold ferric chloride solution gave no coloration. No acridines were formed on heating with formic and hydrochloric acids in glycerol at 175°.

3-Amino-N-acetyldiphenylamine.—3-Nitrodiphenylamine (70 g.) was refluxed with acetic anhydride (100 ml.) and one drop of sulphuric acid for 2 hours. The greater part of the acetic anhydride was removed by distillation and the rest by refluxing with alcohol (100 ml.) for 2 hours. The alcohol was distilled off and the residue recrystallised from a little methanol giving white crystals of *3-nitro-N-acetyldiphenylamine*, m. p. 71°, in 90% yield (Found: C, 65·4; H, 4·9. $C_{14}H_{12}O_3N_2$ requires C, 65·6; H, 4·7%).

Reduction as for 3-nitrodiphenylamine, followed by recrystallisation from alcohol, gave white crystals of *3-amino-N-acetyldiphenylamine*, m. p. 124°, in 95% yield, recrystallising well from benzene and almost insoluble in light petroleum (Found: C, 73·8; H, 6·3. $C_{14}H_{14}ON_2$ requires C, 74·3; H, 6·2%). The cold alcoholic solution gave no colour with ferric chloride.

3-Dimethylaminodiphenylamine.—3-Amino-*N*-acetyldiphenylamine (17 g.), methyl iodide (25 g.; slight excess), methanol (80 ml.), and dried calcium carbonate (8 g.) were heated under reflux for 5 hours in a bath maintained at 40°; during the next 10 hours the temperature was gradually raised to 60°. The product was filtered and the solvent recovered. The residual oil was shaken for 2 hours with *p*-toluenesulphonyl chloride (7·5 g.), ether (20 ml.), and sodium hydroxide (60 ml. of 3N). The alkaline layer (representing primary amine) was run off, counter-extracted with a little ether, and discarded. The combined ethereal fractions were repeatedly extracted with 0·5N-hydrochloric acid. The combined aqueous layers were made alkaline and shaken out thrice with ether. The ether extracts were dried (Na_2SO_4) and taken to dryness. The yield was 5 g. of a pale orange solid which became white on recrystallisation from 40 parts of light petroleum (b. p. 60—90°). The *3-dimethylamino-N-acetyldiphenylamine*, m. p. 97—98° was moderately soluble in boiling water, with gradient, and very soluble in alcohol, acetone, and benzene (Found: C, 75·3; H, 7·1; N, 11·1. $C_{16}H_{18}ON_2$ requires C, 75·6; H, 7·1; N, 11·0%).

This acetyl compound (5 g.) and hydrochloric acid (60 ml. of 7N) were heated for 2 hours at 100°, made alkaline, and refrigerated. The precipitate was powdered and washed well with water, dried in a vacuum, and recrystallised from light petroleum (b. p. 60—90°), the first orange oily drops which separated on cooling being rejected. The *3-dimethylaminodiphenylamine* was obtained in 95% yield as white crystals, m. p. 65—66°, sparingly soluble in water and very soluble in all common organic solvents (Found: C, 78·9; H, 7·6; N, 13·3. $C_{14}H_{16}N_2$ requires, C, 79·2; H, 7·6; N, 13·2%).

This substance gives a pink colour, deepening to purple, with a mixture of equal parts of 3% hydrogen peroxide and 5N-sulphuric acid containing a trace of ferrous sulphate. The *N*-acetyl derivative does not react.

3-Hydroxydiphenylamine.—This has not previously been prepared from a diphenylamine derivative. 3-Amino-*N*-formyldiphenylamine (1·46 g.; Part II) was dissolved in sulphuric acid (40 ml. of 70% v/v) and a solution of sodium nitrite (0·48 g.) in water (7 ml.) added during 17 minutes. Urea (0·1 g.) was then added, and the mixture was kept in a boiling water-bath until effervescence was complete (about 15 minutes), made neutral with sodium hydroxide, and filtered. The solid was extracted with boiling N-sodium hydroxide. The extract was decolorised with carbon, acidified with acetic acid, and refrigerated, giving white crystals of 3-hydroxydiphenylamine, m. p. 81° (lit. 82°), in 35% yield.

3 : 4′-Diaminodiphenylamine.—The following sulphonation of *p*-chloronitrobenzene is preferred to that of Ullmann (*Ber.*, 1909, **42**, 1077) which often becomes violent. *p*-Chloronitrobenzene (210 g.) was heated, in a 1-litre three-necked ground-glass jointed flask provided with thermometer, stirrer, and condenser guarded by a calcium chloride tube, with fuming sulphuric acid (270 ml.; 10% oleum), in an oil-bath kept at 120° for 10 hours. The internal temperature quickly rose to 135° but soon returned to 115°. The yield of 2-chloro-5-nitrobenzenesulphonic acid monohydrate was 297 g. (87% yield), m. p. 165° (sealed). It was converted, by the method of Ullmann and Dahmen (*Ber.*, 1908, **41**, 3746), into 4-nitro-3′-aminodiphenylamine. The more direct synthesis of this from *m*-aminoacetanilide and *p*-bromonitrobenzene gave poor yields. 4-Nitro-3′-aminodiphenylamine was dissolved in alcohol (32 parts), filtered, and reduced with Raney nickel and hydrogen at atmospheric pressure and 70°. The product was filtered by gravity in an atmosphere of carbon dioxide, the alcohol recovered, and the residue distilled, giving *3 : 4′-diaminodiphenylamine* as a white solid in 90% yield, b. p. 251°/2·5 mm. (uncorr.), m. p. 97° (Found: N, 20·9. $C_{12}H_{13}N_3$ requires N, 21·1%).

4-Amino-3′-dimethylaminodiphenylamine.—*m*-Aminodimethylaniline (27 g.), 2-chloro-5-nitrobenzenesulphonic acid monohydrate (51 g.; 1 mol.), sodium carbonate (10·6 g.), and water (400 ml.) were boiled for a few minutes, then calcium carbonate (10 g.) was added and the whole refluxed for 18 hours. The product was precipitated with hydrochloric acid, washed, recrystallised three times from large volumes of water, and dried at 130°, giving *4-nitro-3′-dimethylaminodiphenylamine-2-sulphonic acid hemihydrate* as yellow-brown crystals in 50% yield, m. p. *ca.* 240° (eff., decomp., sealed) (Found: C, 48·1; H, 4·6; N, 12·0. $C_{14}H_{13}O_5N_3S,\frac{1}{2}H_2O$ requires C, 48·5; H, 4·65; N, 12·1%).

This acid (18 g.) was warmed with sulphuric acid (126 ml.; 70% v/v) for 2 hours in a boiling water-bath. The product was cooled, diluted with 3 vols. of water, refrigerated, and the sulphate

filtered off, digested for 5 minutes with hot aqueous ammonia, cooled, and filtered. The cake, which contained 6% of carbon, was recrystallised from alcohol giving *4-nitro-3'-dimethylaminodiphenylamine* as large dark brown plates, m. p. 139°, in 70% yield (Found: C, 65·2; H, 5·9; N, 16·2. $C_{14}H_{15}O_2N_3$ requires C, 65·4; H, 5·8; N, 16·3%).

This compound (10 g.) was reduced in alcohol (25 ml.) with Raney nickel and hydrogen and gave *4-amino-3'-dimethylaminodiphenylamine* as a colourless highly viscous liquid, b. p. 251°/5 mm., in 85% yield (Found: C, 74·6; H, 7·3; N, 18·2. $C_{14}H_{17}N_3$ requires C, 74·0; H, 7·5; N, 18·5%). It absorbs oxygen in the cold and becomes brown.

Diphenylamine-2-aldehyde.—Ethyl diphenylamine-2-carboxylate (20 g.) was refluxed with hydrazine hydrate (6·25 g.) for 10 hours, cooled, shaken out twice with water (10 ml.), then diluted with ether. The solution was dried (Na_2SO_4) and quickly filtered before crystallisation took place. The product was recrystallised from 5 parts of alcohol giving white crystals of *diphenylamine-2-carboxyhydrazide* (12 g.), m. p. 121°, moderately soluble in water (with gradient) and poorly soluble in ether, benzene, or light petroleum (Found: C, 68·1; H, 5·8; N, 18·3. $C_{13}H_{13}ON_3$ requires C, 68·7; H, 5·8; N, 18·5%). It was readily soluble in dilute hydrochloric acid, the solution giving a white precipitate of the azide with sodium nitrite. When the hydrazide was treated with potassium ferricyanide and ammonia, by the general method of Kalb and Gross (*Ber.*, 1926, **59**, 727), no aldehyde was isolated.

This hydrazide (3·15 g.), dissolved in dry pyridine (6 ml.), was treated with *p*-toluenesulphonyl chloride (2·66 g.; 1 mol.) during 5 minutes, the temperature being kept below 60°. The solution was kept at about 20° for 2 hours and then poured into a mixture of ice and dilute hydrochloric acid. The solid was recrystallised from a little alcohol giving white crystals of *diphenylamine-2-carboxy-(β-p-toluenesulphon)hydrazide*, m. p. 186—187°, in 90% yield. It was very soluble in acetone but sparingly soluble in benzene, ether, or boiling water (Found: N, 11·0. $C_{20}H_{19}O_3N_3S$ requires N, 11·0%).

This toluenesulphonyl derivative was degraded by the general method of McFadyen and Stevens (*J.*, 1936, 584) by maintaining 2·6 g. (dissolved in ethylene glycol, 13 ml.) in a bath kept at 160° whilst freshly dehydrated sodium carbonate (1·7 g.; 5 equivs.) was added as fast as foaming allowed. The reaction was allowed to continue for only 75 seconds more, and the product was then cooled, diluted with water (50 ml.), and filtered. The cake was recrystallised from as little methanol as possible giving *diphenylamine-2-aldehyde*, as odourless yellow crystals, m. p. 72·5°, in 80% yield. It was very soluble in *cyclo*hexane, benzene, and alcohol, less soluble in light petroleum, only slightly soluble in water (but with a good gradient), and volatile in steam without decomposition. The solutions were not fluorescent (Found: C, 78·5; H, 5·6; N, 7·2. $C_{13}H_{11}ON$ requires C, 79·1; H, 5·6; N, 7·1%). Like 2-aminobenzaldehyde it has little effect on Schiff's reagent or hot ammoniacal silver nitrate. The pure yellow solution in cold glacial acetic acid is turned red by a trace of sulphuric acid. This indicates that the molecule is accepting a proton on the carbonyl group. The red colour fades as ring closure to acridine occurs, and in 90 minutes at room temperature the solution is yellow again.

3-Nitrodiphenylamine-6-carboxyhydrazide.—Methyl 3-nitrodiphenylamine-6-carboxylate (3·3 g.) and hydrazine hydrate (1·5 g.) did not react under mild conditions and so were heated under reflux for 6 hours in a bath at 130°. The product was stirred with ether (7 ml.) and the crystals filtered off. After recrystallisation from alcohol (16 pts.), red crystals of *3-nitrodiphenylamine-6-carboxyhydrazide*, m. p. 162—163°, were obtained in 40% yield. It is very soluble in benzene and pyridine with gradients (Found: C, 57·0; H, 4·5; N, 20·45. $C_{13}H_{11}O_3N_4$ requires C, 57·3; H, 4·45; N, 20·6%). Hydrochloric acid, of over 0·5N, dissolves it to form an orange solution.

The mother liquors of the nitro-compound yielded white crystals of *5-aminodiphenylamine-2-carboxyhydrazide*, m. p. 189° (50% yield on the original ester), on concentration, partial reduction by hydrazine having occurred during the condensation. This substance can be crystallised from 25 parts of boiling alcohol, and is moderately soluble in benzene, sparingly soluble in ether, and boiling water (Found: C, 63·8; H, 5·8; N, 22·9. $C_{13}H_{14}ON_4$ requires C, 64·4; H, 5·8; N, 23·1%). The solution in dilute hydrochloric acid can be diazotised (orange) and then coupled with β-naphthol (scarlet).

5-Nitrodiphenylamine-2-carboxyhydrazide (2 g.) was dissolved in dry pyridine (15 ml.) and acylated with *p*-toluenesulphonyl chloride as before, giving orange crystals of *5-nitrodiphenylamine-2-carboxy-(β-p-toluenesulphon)hydrazide* which, after being dried at 120° and recrystallised from alcohol, melted at approx. 247° (decomp.) and were insoluble in benzene and ether (Found: N, 13·2. $C_{20}H_{18}O_5N_4S$ requires N, 13·15%).

5-Nitrodiphenylamine-2-carboxyanilide.—This was prepared by the action, in turn, of thionyl chloride and aniline on the free acid in an unsuccessful endeavour to synthesise 3-aminodiphenylamine-6-aldehyde by the general method of Sonn and Muller (*Ber.*, 1919, **52**, 1927), using phosphorus pentachloride followed by stannous chloride. *5-Nitrodiphenylamine-2-carboxyanilide* formed orange crystals from 10 parts of alcohol or 25 parts of benzene, m. p. 153—154° (Found: N, 12·6. $C_{19}H_{15}O_3N_3$ requires N, 12·6%).

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UNIVERSITY OF SYDNEY. [*Received, August 15th*, 1947.]

243. 3 : 4-*Benzfluorenones. Part II. Further Observations on the Effect of Groups on their Fission with Alkali.*

By Fawzy Ghali Baddar and Munir Gindy.

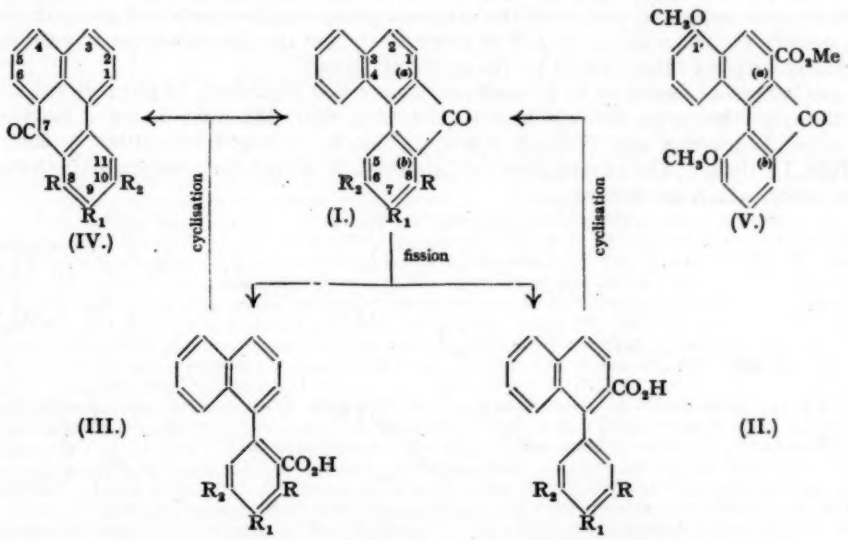
6 : 8-*Dimethyl-* and 6-*methyl*-3 : 4-*benzfluorenone* give, when fused with alkali, mixtures of 1-m-5′-*xylylnaphthalene*-2- and -4′-*carboxylic acid*, and 1-m-*tolylnaphthalene*-2- and -4′-*carboxylic acid* respectively. In both cases the 4′-acid is isolated in a greater proportion. 7-*Methyl*-3 : 4-*benzfluorenone*, however, gives on fission a mixture of the 2- and 4′-*carboxylic acids*, in the ratio of about 3 : 2 respectively.

1′ : 5-Dimethoxy-3 : 4-benzfluorenone-1-carboxylic acid gives, when similarly treated, 5 : 6′-*dimethoxy*-1-*phenylnaphthalene*-2′ : 3-*dicarboxylic acid* only.

These results support the view that the direction of fission of 3 : 4-benzfluorenones by fused potassium hydroxide is influenced by the polar character of the substituent (cf. Part I, *J.*, 1944, 450).

In Part I (*loc. cit.*) the authors concluded, from a study of the effect of electron-attracting groups (*e.g.*, CO_2H) on the mode of fission of 3 : 4-benzfluorenones, that the direction of cleavage is governed by the polar character of the group rather than its steric effect.

To prove the validity of this statement, the study has been extended to 3 : 4-benzfluorenones substituted either by electron-repelling groups or by both electron-repelling and electron-attracting groups. The following examples were, therefore, prepared and then cleaved with alkali.



(R and R_2 = H or Me; R_1 = H, Me, or NO_2.)

6 : 8-*Dimethyl*-3 : 4-*benzfluorenone* (I; R and R_2 = Me, R_1 = H) was prepared by condensation of 5-iodo-*m*-xylene with methyl 1-bromo-2-naphthoate in presence of copper-bronze, and ring closure of the **1-m-5′-*xylylnaphthalene*-2-*carboxylic acid*** (II; R_1 = H, R and R_2 = Me) so formed.

Methyl 3-bromo-*p*-toluate was condensed with α-iodonaphthalene in presence of copper-bronze to give **1-m-*tolylnaphthalene*-4′-*carboxylic acid*** (III; R and R_1 = H, R_2 = Me). This gave on cyclisation a mixture of **6-*methyl*-3 : 4-*benzfluorenone*** (I; R and R_1 = H, R_2 = Me) and 10-methyl*meso*benzanthrone (IV; R and R_1 = H, R_2 = Me).

1-p-*Tolylnaphthalene*-2-*carboxylic acid* (II; R and R_2 = H, R_1 = Me), prepared by the condensation of *p*-iodotoluene and methyl 1-bromo-2-naphthoate, gave on ring closure **7-*methyl*-3 : 4-*benzfluorenone*** (I; R and R_2 = H, R_1 = Me).

Methyl 1′ : 5-dimethoxy-3 : 4-benzfluorenone-1-carboxylate (V) was prepared by the action of aluminium chloride on 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride in nitrobenzene, followed by esterification with diazomethane in dioxan (cf. Baddar, *J.*, 1947, 224).

α-Iodonaphthalene condensed similarly with methyl 2-iodo-5-nitrobenzoate to give **4′-*nitro*-1-*phenylnaphthalene*-2′-*carboxylic acid***, which was cyclised to a mixture of **7-*nitro*-3 : 4-*benzfluorenone*** (I; R and R_2 = H, R_1 = NO_2) and 9-nitro*meso*benzanthrone (IV; R and R_2 = H, R_1 = NO_2).

Alkaline Fission of 3 : 4-*Benzfluorenones.*—6 : 8-Dimethyl- and 6-methyl-3 : 4-benzfluorenone gave on fission with alkali mixtures of 1-*m*-5′-xylylnaphthalene-2- and -4′ (III; R_1 = H, R and R_2 = Me)-carboxylic acid, and 1-m-*tolylnaphthalene*-2- (II; R_1 and R_2 = H, R = Me) and 1-*m*-tolylnaphthalene-4′-carboxylic acid respectively. In both cases the 2- and the 4′-carboxylic acid were obtained in the ratio of about 2 : 3 respectively. 7-Methyl-3 : 4-benzfluorenone, however, gave on fission a mixture of 1-*p*-tolylnaphthalene-2- and -3′(III; R and R_2 = H, R_1 = Me)-carboxylic acid in the ratio of about 3 : 2 respectively.

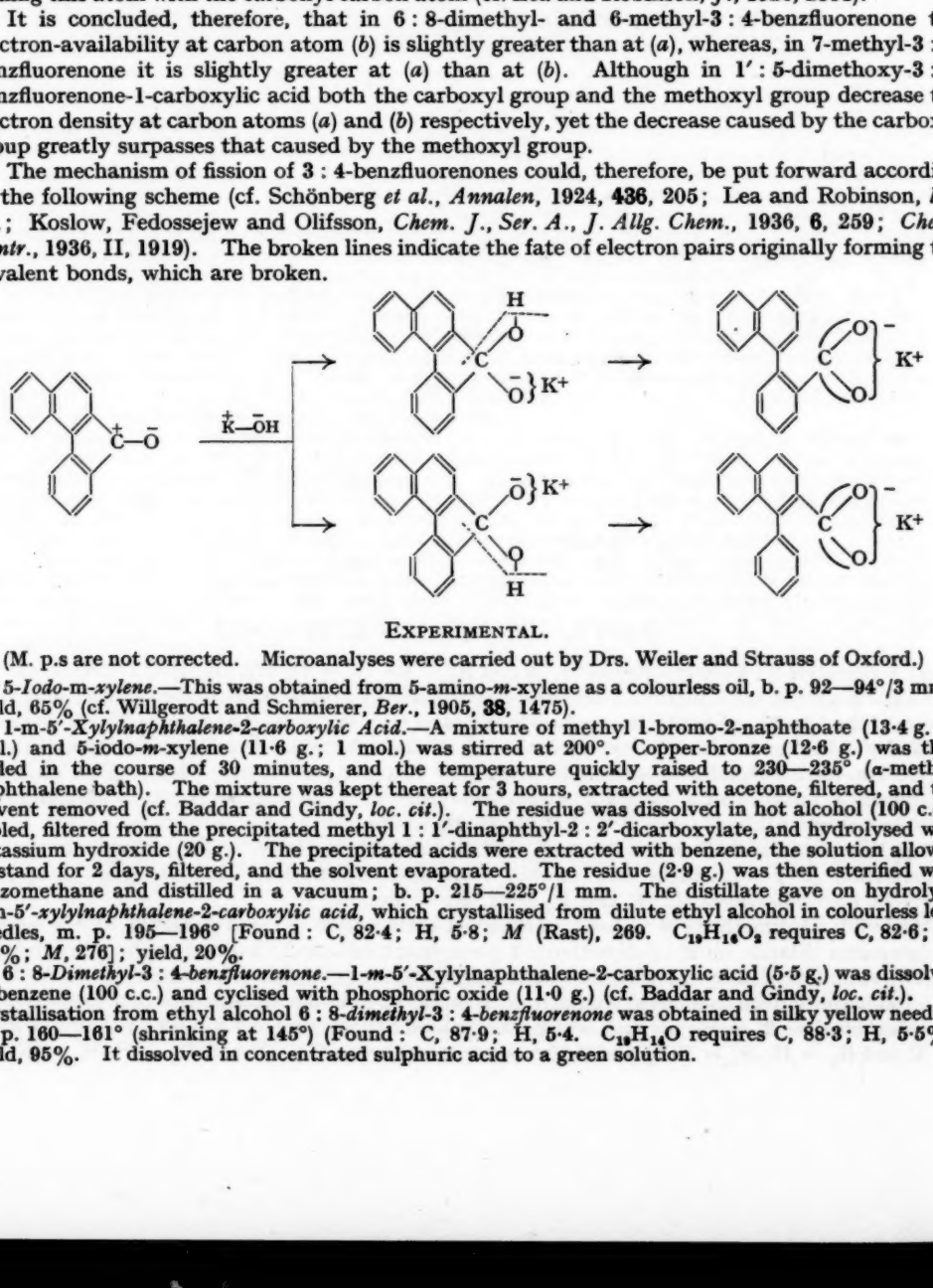
When 1′ : 5-dimethoxy-3 : 4-benzfluorenone-1-carboxylic acid (or its ester) was similarly treated it gave only 5 : 6′-*dihydroxy*-1-*phenylnaphthalene*-2′ : 3-*dicarboxylic acid*, owing to demethylation during fission. Its *dimethyl ether* was not identical with 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic acid (cf. Baddar, *loc. cit.*); it gave, however, on decarboxylation 2′ : 5-dimethoxy-1-phenylnaphthalene.

Under similar or milder conditions 7-nitro-3 : 4-benzfluorenone resinified.

These results support the authors' conclusion in Part I (*loc. cit.*), that the factors which operate to determine the point of cleavage of 3 : 4-benzfluorenones are of polar origin, depending to a great extent on the electron density at carbon atoms (*a*) and (*b*) in (I) and (V). The greater the density of electrons at any of the two carbon atoms (*a*) and (*b*) the more stable is the bond linking this atom with the carbonyl carbon atom (cf. Lea and Robinson, *J.*, 1926, 2351).

It is concluded, therefore, that in 6 : 8-dimethyl- and 6-methyl-3 : 4-benzfluorenone the electron-availability at carbon atom (*b*) is slightly greater than at (*a*), whereas, in 7-methyl-3 : 4-benzfluorenone it is slightly greater at (*a*) than at (*b*). Although in 1′ : 5-dimethoxy-3 : 4-benzfluorenone-1-carboxylic acid both the carboxyl group and the methoxyl group decrease the electron density at carbon atoms (*a*) and (*b*) respectively, yet the decrease caused by the carboxyl group greatly surpasses that caused by the methoxyl group.

The mechanism of fission of 3 : 4-benzfluorenones could, therefore, be put forward according to the following scheme (cf. Schönberg *et al.*, *Annalen*, 1924, **436**, 205; Lea and Robinson, *loc. cit.*; Koslow, Fedossejew and Olifsson, *Chem. J., Ser. A., J. Allg. Chem.*, 1936, **6**, 259; *Chem. Zentr.*, 1936, II, 1919). The broken lines indicate the fate of electron pairs originally forming the covalent bonds, which are broken.



Experimental.

(M. p.s are not corrected. Microanalyses were carried out by Drs. Weiler and Strauss of Oxford.)

5-*Iodo*-m-*xylene.*—This was obtained from 5-amino-*m*-xylene as a colourless oil, b. p. 92—94°/3 mm.; yield, 65% (cf. Willgerodt and Schmierer, *Ber.*, 1905, **38**, 1475).

1-m-5′-*Xylylnaphthalene*-2-*carboxylic Acid.*—A mixture of methyl 1-bromo-2-naphthoate (13·4 g.; 1 mol.) and 5-iodo-*m*-xylene (11·6 g.; 1 mol.) was stirred at 200°. Copper-bronze (12·6 g.) was then added in the course of 30 minutes, and the temperature quickly raised to 230—235° (α-methylnaphthalene bath). The mixture was kept thereat for 3 hours, extracted with acetone, filtered, and the solvent removed (cf. Baddar and Gindy, *loc. cit.*). The residue was dissolved in hot alcohol (100 c.c.), cooled, filtered from the precipitated methyl 1 : 1′-dinaphthyl-2 : 2′-dicarboxylate, and hydrolysed with potassium hydroxide (20 g.). The precipitated acids were extracted with benzene, the solution allowed to stand for 2 days, filtered, and the solvent evaporated. The residue (2·9 g.) was then esterified with diazomethane and distilled in a vacuum; b. p. 215—225°/1 mm. The distillate gave on hydrolysis 1-m-5′-*xylylnaphthalene*-2-*carboxylic acid*, which crystallised from dilute ethyl alcohol in colourless long needles, m. p. 195—196° [Found: C, 82·4; H, 5·8; *M* (Rast), 269. $C_{19}H_{16}O_2$ requires C, 82·6; H, 5·8%; *M*, 276]; yield, 20%.

6 : 8-*Dimethyl*-3 : 4-*benzfluorenone.*—1-*m*-5′-Xylylnaphthalene-2-carboxylic acid (5·5 g.) was dissolved in benzene (100 c.c.) and cyclised with phosphoric oxide (11·0 g.) (cf. Baddar and Gindy, *loc. cit.*). On crystallisation from ethyl alcohol 6 : 8-*dimethyl*-3 : 4-*benzfluorenone* was obtained in silky yellow needles, m. p. 160—161° (shrinking at 145°) (Found: C, 87·9; H, 5·4. $C_{19}H_{14}O$ requires C, 88·3; H, 5·5%); yield, 95%. It dissolved in concentrated sulphuric acid to a green solution.

Fusion of 6 : 8-*Dimethyl*-3 : 4-*benzfluorenone with Potassium Hydroxide.*—The benzfluorenone (1 part) was added during 10 minutes to fused potassium hydroxide (7 parts) at 230—235° and kept thereat with frequent stirring for a further 15 minutes. The alkaline aqueous extract was filtered and acidified. The dry acids were esterified with ethereal diazomethane and distilled in a vacuum; b. p. 220—230°/3 mm. The ester gave on hydrolysis with alcoholic potassium hydroxide a mixture of acids which melted at 60—70°. The crude acids (0·3 g.) were refluxed with 50% aqueous methyl alcohol (150 c.c.) and filtered hot. This solution precipitated on standing overnight in the ice-chest a crystalline acid (0·1 g.), m. p. 187—191°, raised on recrystallisation to 194—195°, undepressed on admixture with an authentic specimen of 1-*m*-xylylnaphthalene-2-carboxylic acid.

The mother liquor was evaporated under reduced pressure at room temperature until it became turbid. On standing overnight in the ice-chest, 1-m-5′-*xylylnaphthalene-4′-carboxylic acid* (0·14 g.) separated in colourless cubes, m. p. 159—161°, raised on recrystallisation from dilute methyl alcohol to 164—165°, depressed on admixture with authentic 1-*m*-5′-xylylnaphthalene-2-carboxylic acid to 138—155° (Found: C, 82·6; H, 5·9. $C_{19}H_{16}O_2$ requires C, 82·6; H, 5·8%). It gave with concentrated sulphuric acid a dirty brown colour with a green fluorescence indicating the formation of a mixture of a *meso*benzanthrone and a 3 : 4-benzfluorenone.

Ethyl 1-*Bromo*-2-*naphthoate.*—The crude acid (43 g., prepared according to Boyes, Grieve, and Rule, *J.*, 1938, 1833) was refluxed with absolute ethyl alcohol (300 c.c.) and concentrated sulphuric acid (72 c.c.) for 10 hours. The product was crystallised from light petroleum (b. p. 30—50°), from which ethyl 1-bromo-2-naphthoate separated in colourless cubes, m. p. 36—37° (Found: Br, 28·4. $C_{13}H_{11}O_2Br$ requires Br, 28·6%); yield, 90%.

1-m-*Tolylnaphthalene-2-carboxylic Acid.*—A mixture of *m*-iodotoluene (13·3 g.; 1 mol.) and ethyl 1-bromo-2-naphthoate (17·0 g.; 1 mol.) was stirred at 200°, treated with copper-bronze (15·3 g.), and worked up as in the case of 1-*m*-5′-xylylnaphthalene-2-carboxylic acid. The product was esterified with diazomethane and distilled in a vacuum. The fraction boiling below 180°/3 mm. was rejected; that boiling at 185—187°/3 mm. was directly hydrolysed with alcoholic potassium hydroxide. The acid was crystallised from dilute alcohol to give 1-m-*tolylnaphthalene-2-carboxylic acid* in colourless cubes, m. p. 164—165° (Found: C, 82·3; H, 5·3. $C_{18}H_{14}O_2$ requires C, 82·4; H, 5·4%); yield, 23·7%.

Ring Closure of 1-m-*Tolylnaphthalene-2-carboxylic Acid.*—The acid was cyclised as usual with phosphoric oxide in benzene. The product was repeatedly crystallised from alcohol to give a eutectic inseparable mixture of 6- and 8-methyl-3 : 4-benzfluorenone in fine orange needles, m. p. 112—118° (Found: C, 88·0; H, 5·2. Calc. for $C_{18}H_{12}O$: C, 88·5; H, 5·0%); yield, quantitative.

3-*Bromo*-p-*toluic Acid*—3-Bromo-*p*-toluidine (46·5 g.) was dissolved in dilute sulphuric acid (25 c.c. acid and 80 c.c. water), and diazotised as usual with sodium nitrite (19 g., in 30 c.c. water). The filtered diazonium salt solution was added portionwise (15 minutes) to a cuprous cyanide solution, obtained by mixing solutions of copper sulphate (124·5 g. of $CuSO_4.5H_2O$ in 500 c.c. of water) and sodium cyanide (110 g. in 280 c.c. of water). The mixture was left overnight then steam distilled. 3-Bromo-4-cyanotoluene was obtained in 68% yield as a colourless solid, m. p. 46—47°. This was hydrolysed by refluxing it for 4 hours with 50% v/v sulphuric acid. The product, precipitated on dilution, was filtered off, dissolved in sodium carbonate solution, and the unchanged parent substance (3 g.) extracted with ether. 3-Bromo-*p*-toluic acid crystallised from water or very dilute alcohol in colourless feathery needles, m. p. 143°; yield, 76%. Claus and Kunath (*J. pr. Chem.*, 1889, **39**, 486) give m. p. 140°. The crude acid was esterified with diazomethane, and the *methyl* ester was distilled in a vacuum, b. p. 167—168°/38 mm., 161—164°/32 mm., to a colourless mobile oil (Found: C, 47·6; H, 4·0; Br, 35·7. $C_9H_9O_2Br$ requires C, 47·2; H, 4·0; Br, 34·9%).

1-m-*Tolylnaphthalene-4′-carboxylic Acid.*—A mixture of 3-bromo-*p*-toluic ester (11·5 g.; 1 mol.) and α-iodonaphthalene (12·7 g.; 1 mol.) was stirred at 180°, and copper-bronze (12·5 g.) was added portionwise during ½ hour. The temperature was then raised to 205—210° (nitrobenzene bath) and the reaction continued for a further 4 hours. The product was worked up as described above, and the precipitated acids were dried and extracted with benzene (to remove the insoluble ditolyldicarboxylic acid). The acid residue was esterified with diazomethane, or with thionyl chloride and methyl alcohol, and fractionally distilled at 40 mm. The fraction of b. p. <200° was rejected; that of b. p. 235—245° gave on hydrolysis 1-m-*tolylnaphthalene-4′-carboxylic acid*, which crystallised from dilute alcohol in colourless needles, m. p. 176—177° (Found: C, 82·6; H, 5·2. $C_{18}H_{14}O_2$ requires C, 82·4; H, 5·4%); yield, 33%. It gave with concentrated sulphuric acid a dark red colour with a green fluorescence.

6-*Methyl*-3 : 4-*benzfluorenone and* 10-*Methyl*meso*benzanthrone.*—The above acid was cyclised in the usual way to a dark orange product, m. p. 120—130°. This was fractionally crystallised from methyl alcohol, from which 6-*methyl*-3 : 4-*benzfluorenone* separated at first in long feathery orange needles, m. p. 160—161°; it gave with concentrated sulphuric acid a green non-fluorescent solution (Found: C, 87·8; H, 4·6. $C_{18}H_{12}O$ requires C, 88·5; H, 4·95%); yield, 50%. The mother liquor deposited on further cooling 10-methyl*meso*benzanthrone in light yellow cubic crystals, m. p. 152—154°, raised after two crystallisations from acetone to 159° (Found: C, 88·3; H, 4·63. Calc. for $C_{18}H_{12}O$: C, 88·5; H, 4·95%); yield, 50%. Fieser and Martin (*J. Amer. Chem. Soc.*, 1936, **58**, 1444) give m. p. 158—159°. The pure benzanthrone could be obtained in a 50% yield by heating the cyclisation mixture with 90% v/v sulphuric acid at 100° for 1 hour. Under these conditions the 3 : 4-benzfluorenone was completely sulphonated.

Fission of 6-*Methyl*-3 : 4-*benzfluorenone.*—The ketone (1 g.) was fused with potassium hydroxide as described above, and the resulting acids were purified by esterification and distillation in a vacuum. The fraction, b. p. 230—250°/1 mm., was hydrolysed, and the precipitated acids filtered off (0·3 g.). The separation of the constituents by crystallisation was practically impossible, and the relative amounts of the two acids in the fission product was determined by taking advantage of (*a*) the fact that 1-*m*-tolylnaphthalene-2-carboxylic acid gave on ring closure a mixture of 6- and 8-methyl-3 : 4-benzfluorenone, whereas 1-*m*-tolylnaphthalene-4′-carboxylic acid gave a mixture of equimolecular amounts of 6-methyl-3 : 4-benzfluorenone and 10-methyl*meso*benzanthrone, and (*b*) that whereas 6- and 8-methyl-3 : 4-benzfluorenone were completely sulphonated on being heated with 90% v/v sulphuric acid for 1 hour at

100°, 10-methyl*meso*benzanthrone was recovered totally unchanged. It seemed possible, therefore, that the percentage composition of the fission product could be determined by cyclising it, then heating a known weight with 90% sulphuric acid, and estimating the amount of 10-methyl*meso*benzanthrone. The cyclised product (0·2 g.) was heated with 90% sulphuric acid (20 c.c.) at 100° for 1 hour, poured into water, and the precipitated solid extracted with pure benzene. The solution was washed, filtered, and the solvent evaporated (constant weight), leaving the unsulphonated 10-methyl*meso*benzanthrone (0·06 g., 30%). The ratio of 1-*m*-tolylnaphthalene-4′-carboxylic acid to 1-*m*-tolylnaphthalene-2-carboxylic acid was then calculated to be: $2 \times 30 : (100 - 2 \times 30) = 3 : 2$.

1-p-*Tolylnaphthalene-2-carboxylic Acid.*—A mixture of *p*-iodotoluene (21 g.; 1 mol.) and methyl 1-bromo-2-naphthoate (25·5 g.; 1 mol.) was stirred at 200°, and copper-bronze (21 g.) added portionwise during ½ hour. The temperature was then quickly raised to 230—235°, and the reaction continued for a further 3 hours. The product was worked up as usual, and the dry acids extracted with benzene. The benzene solution was left for 2 days, and then filtered from the precipitated dinaphthyldicarboxylic acid. The acid was purified through its ester (b. p. 194—196°/1 mm.), and then crystallised from benzene–light petroleum (b. p. 40—70°), from which 1-p-*tolylnaphthalene-2-carboxylic acid* separated in monoclinic crystals, m. p. 183—184° (Found: C, 82·7; H, 5·3. $C_{18}H_{14}O_2$ requires C, 82·4; H, 5·4%); yield, 33%. It was soluble in alcohol, ether, and benzene, sparingly so in light petroleum. It gave with concentrated sulphuric acid a green non-fluorescent solution.

7-*Methyl*-3 : 4-*benzfluorenone.*—The above acid was cyclised quantitatively with phosphoric oxide in benzene. The product was repeatedly crystallised from alcohol, from which 7-*methyl*-3 : 4-*benzfluorenone* was obtained in orange needles, m. p. 127—128°, soluble in ether and benzene, sparingly soluble in light petroleum. It gave with concentrated sulphuric acid a green non-fluorescent solution. When the ketone was distilled at 230—240°/5 mm., and the distillate crystallised from alcohol, it gave light yellow needles with the same m. p. which turned orange on heating (100—110°) (Found: C, 88·4; H, 5·0. $C_{18}H_{12}O$ requires C, 88·5; H, 4·95%).

Fusion of 7-*Methyl*-3 : 4-*benzfluorenone with Alkali.*—7-Methyl-3 : 4-benzfluorenone (1 g.) was fused with potassium hydroxide, and worked up as above. The resulting acids were esterified, distilled (b. p. 230—240°/1 mm.), and hydrolysed. The dry acids (0·4 g., m. p. 65—75°) were dissolved in the least amount of 75% v/v aqueous methyl alcohol, left for 1 hour at room temperature (30—35°), and the crystals filtered off; these were proved to be 1-*p*-tolylnaphthalene-2-carboxylic acid. The mother liquor was evaporated to dryness, the residue crystallised from the least amount of 70% methyl alcohol, and the product filtered off after 1—2 hours. Recrystallisation from dilute methyl alcohol gave 1-p-*tolylnaphthalene-3′-carboxylic acid* in colourless needles, m p. 161—162°, depressed to 135—145° on admixture with 1-*p*-tolylnaphthalene-2-carboxylic acid (Found: C, 82·1; H, 5·4. $C_{18}H_{14}O_2$ requires C, 82·4; H, 5·4%). It gave with concentrated sulphuric acid a dirty red solution with a green fluorescence. The -2- and the -3′-carboxylic acid were obtained in the ratio of about 3 : 2 respectively.

Methyl 1′ : 5-*Dimethoxy*-3 : 4-*benzfluorenone*-1-*carboxylate.*—The cold suspension of the acid (20 g.) (cf. Baddar, *loc. cit.*) in dioxan (200 c.c.) was treated with an ethereal solution of diazomethane, and the mixture kept at room temperature (15—20°) for 24 hours. The precipitated ester was washed with hot ammonia solution, then crystallised from dioxan–alcohol, from which methyl 1′ : 5-dimethoxy-3 : 4-benzfluorenone-1-carboxylate separated in red prismatic needles, m. p. 190·5—191·5° (cf. Baddar, *loc. cit.*); yield, 63·9%.

Alkaline Fission of Methyl 1′ : 5-*Dimethoxy*-3 : 4-*benzfluorenone*-1-*carboxylate.*—The above ester (2 g.) was similarly fused with potassium hydroxide; a copious evolution of gas was observed which subsided after about 15 minutes. The precipitated acid (1·7 g.) was crystallised from water, from which 5 : 6′-*dihydroxy*-1-*phenylnaphthalene*-2′ : 3-*dicarboxylic acid* separated in brownish-yellow clusters, m. p. 283—285° (Found: C, 60·7; H, 4·56. $C_{18}H_{12}O_6,2H_2O$ requires C, 60·0; H, 4·48%). On dehydration, by heating at 200—210° in a vacuum, the m. p. was raised to 294—296° (Found: C, 66·4; H, 4·0. $C_{18}H_{12}O_6$ requires C, 66·6; H, 3·7%).

The acid (1·6 g.), which was proved to contain no methoxyl groups (demethylation during fusion), was methylated by refluxing its suspension in dry acetone (60 c.c.) with methyl iodide (6 c.c.) and potassium carbonate (7 g.) for 3 hours; then a further quantity of methyl iodide (4 c.c.) was added, and the refluxing continued for a further 8 hours. The acetone was removed, and the residue extracted with benzene and washed with sodium hydroxide solution. The solvent was evaporated, and the residue hydrolysed to the free acid, which was refluxed with acetic anhydride for 10 minutes. The product was precipitated with water, filtered off, and extracted with dilute cold sodium hydroxide solution (all dissolved), which indicated that the fission product was free from 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic acid. The alkaline solution (charcoal) was acidified, and the precipitated acid was crystallised from glacial acetic acid, from which 5 : 6′-dimethoxy-1-phenylnaphthalene-2′ : 3-dicarboxylic acid separated in colourless small cubes, m. p. above 340°. It was best identified as its dimethyl ester, which was prepared by treating a dioxan solution of the acid with an ethereal solution of diazomethane. On crystallisation from ether, followed by benzene–light petroleum (b. p. 60—70°), the *methyl* ester was obtained in colourless rods, m. p. 170—171°, depressed on admixture with a specimen of methyl 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylate to 125—140° (cf. Baddar, *loc. cit.*) (Found: C, 69·0; H, 5·0. $C_{22}H_{20}O_6$ requires C, 69·4; H, 5·3%). It gave with concentrated sulphuric acid a clear red solution with a brown fluorescence.

2′ : 5-*Dimethoxy*-1-*phenylnaphthalene.*—A mixture of the above acid (0·5 g.), quinoline (3 c.c.), and copper-bronze (0·1 g.) was stirred at 200—210° for 3 hours. The product was worked up as usual (cf. Baddar, *loc. cit.*), distilled in a vacuum, and then crystallised from methyl alcohol to give 2′ : 5-dimethoxy-1-phenylnaphthalene in monoclinic crystals, m. p. 95—96°, undepressed on admixture with an authentic specimen.

4′-*Nitro*-1-*phenylnaphthalene*-2′-*carboxylic Acid.*—A mixture of methyl 2-iodo-5-nitrobenzoate (5 g.; 1 mol.) and α-iodonaphthalene (5 g.; 1·2 mol.) was stirred and heated at 180°, and copper-bronze (4 g.) added slowly during ½ hour. The temperature was then raised to 200—210°, and the reaction continued

for a further 2 hours. The product was extracted in the usual way, and hydrolysed by being boiled with a mixture of 80% acetic acid (65 c.c.) and concentrated sulphuric acid (5 c.c.) for 2 hours. The acids were precipitated with ice, filtered off, and extracted with sodium hydrogen carbonate solution (charcoal). The precipitated acids were extracted with benzene and filtered from the insoluble dinitrodiphenic acid. On crystallisation from alcohol *4'-nitro-1-phenylnaphthalene-2'-carboxylic acid* separated in yellow needles, m. p. 195—196° (Found: C, 69·8; H, 3·7; N, 5·0. $C_{17}H_{11}O_4N$ requires C, 69·6; H, 3·8; N, 4·8%). It gave with concentrated sulphuric acid a brown colour with a green fluorescence.

2-Iodo-5-nitrobenzoic acid was prepared according to Goldstein and Grampoloff (*Helv. Chim. Acta*, 1930, **13**, 310) with only half the amount of the nitrating mixture, and heating for 1½—2 hours at 140—150°.

Ring-closure of 4'-Nitro-1-phenylnaphthalene-2'-carboxylic Acid.—The above acid was ring-closed with phosphoric oxide in benzene to a mixture of a 3 : 4-benzfluorenone and a *meso*benzanthrone. On crystallisation from glacial acetic acid, *7-nitro-3 : 4-benzfluorenone* was obtained in red needles, m. p. 265—266° (Found: N, 5·2. $C_{17}H_9O_3N$ requires N, 5·1%).

The 9-nitro*meso*benzanthrone was present in a very small proportion and did not crystallise out from the acetic acid.

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244. *The Adaptation of Bacteria to Acridine Derivatives. The Influence of pH.*

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Increasing hydrogen-ion concentration diminishes the bacteriostatic action of proflavine (2 : 8-diaminoacridine) on *Bact. lactis aerogenes*, as found by Albert *et al.* for various other acridines and *Bact. coli*. The concentration of the compound, $m_{\Delta L}$, required to produce a standard increase in the lag is related to $[H^+]$ by the equation $m_{\Delta L}/[H^+]^n = \text{constant}$. Changes in pH in the range studied do not affect the lag of cells in the absence of the compound, or according to the work of others, the dissociation of the proflavine which is virtually complete.

The exponent n is about 0·52 for normal cells. If cells are trained to resist proflavine at a concentration $\overline{m}$, n remains nearly constant over the range $\overline{m} = 0$ to $\overline{m} = 100$, and then rises to about 0·85.

These and other results suggest that quantitative changes in the cell enzymes occur on training to lower concentrations of proflavine. On training to higher concentration qualitative changes are superimposed.

THE growth of bacterial cells in the presence of compounds which inhibit reactions involving auto-synthetic enzymes must often alter the material balance of the cell. This effect of the compound is not infrequently compensated by the resulting changes in the balance of the cell processes, so that the growth characteristics return to normal. Adaptation is then said to occur.

The problem of adaptation can be studied from various angles; in the following the effect of one important factor, the hydrogen-ion concentration, is investigated. The specific example chosen is the bacteriostatic action of proflavine (2 : 8-diaminoacridine) and of 5-aminoacridine upon *Bact. lactis aerogenes*.

The main effects of the addition of proflavine to the nutrient medium have already been described (Davies, Hinshelwood, and Pryce, *Trans. Faraday Soc.*, 1944, **40**, 397). On repeated subculture in the presence of proflavine, the cells adapt themselves with a marked reduction in the length of the lag phase. For any given strain there is a characteristic relation between lag (L) and concentration (m). When any strain has been repeatedly subcultured at a given concentration ($\overline{m}$), the m–L curve is displaced along the concentration axis by an amount which, over a certain range, is equal to $\overline{m}$.

Theoretical accounts of training phenomena have been based on the alternative ideas of natural selection of resistant strains previously present, of enzyme modification during the training process, and of change in the mode of cell division (or in some hereditary cell mechanism). The last two theories imply that actual modifications of individual cells occur and this is supported by the bulk of the evidence (Hinshelwood, *Biol. Reviews*, 1944, **19**, 158) although the action of selection must be superimposed on other mechanisms once a mixed population of modified and unmodified cells arises. Enzyme modifications in the individual cells may occur either (*a*) by quantitative changes in existing mechanisms and alterations of the enzyme balance in the cells, or (*b*) by qualitative modifications in enzyme surfaces involving changes unfavourable to attack by the toxic substance.

Theories of type (*a*) have been employed to interpret many of the facts of proflavine training (Davies, Hinshelwood, and Pryce, *loc. cit.*). The compound is regarded as impeding the utilisation of an intermediate substance required by a group of enzymes. Training is then envisaged as the quantitative expansion relative to these enzymes of the enzyme systems

producing the intermediate. Modifications of type (*b*) are, however, not excluded, and it is of some interest to discover a means of estimating their relative importance.

Albert *et al.* (*Brit. J. Exp. Path.*, 1945, **26**, 160) have accumulated a large body of evidence which indicates that the charged cation is the active bacteriostatic agent in solutions of the acridines. For example, it was found that the activity was greater the higher the degree of dissociation at a given pH. In the same paper an account is given of a study of the effect of changes in hydrogen-ion concentration on the antibacterial activity of several acridines towards *Bact. coli*. As the hydrogen-ion concentration increased an increasing concentration of acridinium ion was required to prevent any growth within 48 hours. The percentage of compound present as cation at a given pH was known from potentiometric measurements (Albert and Goldacre, *J.*, 1943, 454). The graphs of the logarithm of the inhibitory concentrations of the various cations against the pH of the media were found to constitute a series of close, parallel straight lines. Albert suggested that competition was occurring between the acridine cations and the hydrogen ions for the same positions on certain enzyme surfaces within the cell. (The drugs used did not include proflavine.)

In the light of Albert's work it is of interest to inquire how the relative competitive powers of hydrogen ions and acridinium ions will be altered during adaptation. Any changes will be reflected in corresponding changes in the slope and shape of the lines. This suggests a possible method of distinguishing between modifications of types (*a*) and (*b*), occurring during training, of the enzymes upon the surfaces of which the competition is considered to be located.

The purpose of the present study was to investigate the competition between proflavine and hydrogen ions in the growth of a series of trained strains of *Bact. lactis aerogenes*, with special reference to any information this might provide about the nature of the adaptive process.

Experimental Methods.—The experimental techniques of sterilisation, inoculation, culturing in liquid medium and determination of growth characteristics were similar to those previously used in this laboratory and described elsewhere (Davies, Hinshelwood, and Pryce, *loc. cit.*).

The media contained glucose, magnesium sulphate, ammonium sulphate, and phosphate buffer of appropiate pH, together with varying amounts of proflavine sulphate. They were maintained at 40·0° ± 0·05° and were aerated with sterile air, saturated with water vapour.

The pH of the complete solution was measured in a suitably standardised Cambridge glass-electode pH meter (± 0·02 unit of pH). The main cause of variability of pH in a given buffer was the hydrolysis of the proflavine sulphate. To allow for this, the pH was measured over a particular range of proflavine concentrations and the average taken. The mean variation about this averge was ± 0·02, the maximum being ± 0·04.

Before proceeding with the main experiments, it was necessary to determine how hydrogen ions affected the magnitude of the lag in the absence of the proflavine. The curves relating the lag with the age of the parent culture proved to be independent of pH.

Lag–concentration curves of normal cells were then determined at various hydrogen-ion concentrations in the presence of proflavine. A plot of pH against the logarithm of the concentration ($m_{\Delta L}$) required to produce various assigned increases of the lag (ΔL) gave a series of parallel straight lines. That the slope of these lines depended very little on the particular value of lag chosen (provided it exceeded about 1000 minutes) was of practical importance since the slopes could be obtained without always measuring the very long lags of 5000—6000 minutes. The standard slope was found to be 0·52.

As will be discussed later, the constancy or otherwise of this slope (n) gives some help in distinguishing between the two possibilities of qualitative and quantitative enzyme modifications during adaptation.

Similar experiments were therefore performed with cells trained to given proflavine concentrations ($\overline{m}$). A family of lag–concentration curves was obtained for different pH's. Plots of log ($m_{\Delta L}$) against the corresponding pH gave straight lines for each strain. The value of the slope (n) remained constant at 0·5—0·6 until a training concentration of about 100 mg./l. was reached, when there was an increase to 0·8—0·9.

Effect of Varying pH upon the Lag in the Acridine-free Medium.—In order to determine how varying hydrogen-ion concentration influenced the lag–age curve (Lodge and Hinshelwood, *J.*, 1943, 213), a culture in a standard glucose–phosphate–ammonium sulphate medium (pH = 7·12) was sub-cultured at intervals into three media of pH 6·17, 7·25, and 7·56, respectively. Only one lag–age curve could be drawn through the three sets of determinations and this had the usual shape (Fig. 1).

This result shows that any effect of hydrogen ions on the lag in the presence of the acridine must be due to changes in the influence of the drug itself.

Influence of pH on the Lag of Untrained Cells in the Presence of the Acridine.—Preliminary experiments showed that the concentration of proflavine or of 5-aminoacridine required to produce a given lag was higher the lower the pH of the medium.

FIG. 1.

Influence of pH on lag–age relation.

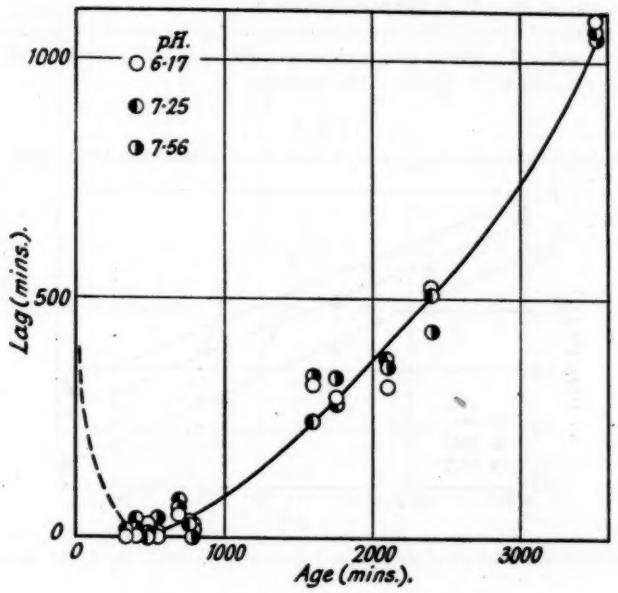
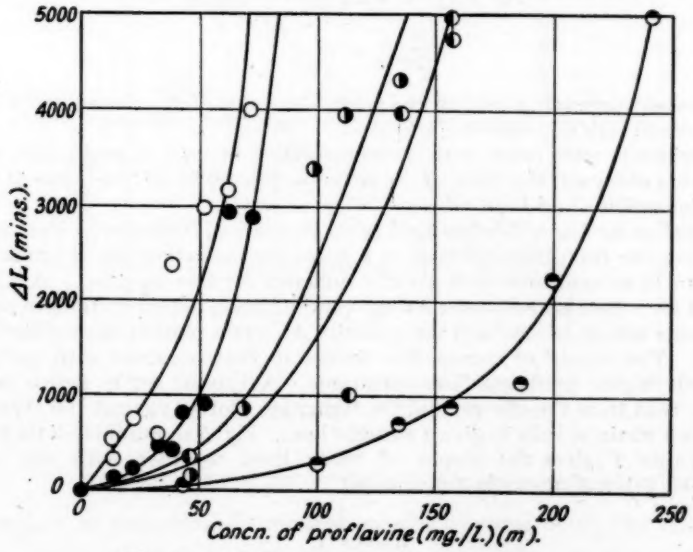


FIG. 2.

Lag–concentration curves for normal cells in proflavine at varying pH.



pH: ○ 7·67, ● 7·20(5), ◑ 6·74, ◐ 6·43(5), ◓ 6·11.

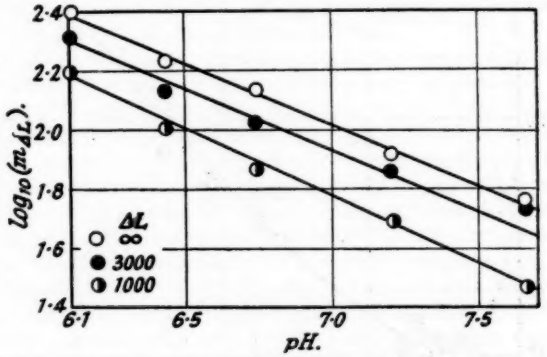
For the main series of experiments inocula were taken from a parent at the end of the logarithmic phase and transferred to series of media with a range of proflavine concentrations and a range of pH values. The lag, L_0, of these inocula was nearly zero for the acridine-free

medium, but to eliminate small variations the quantity used for plotting was $\Delta L = L - L_0$, L being the measured lag, in minutes, in any given medium. From the results the ΔL–concentration curves of normal cells in media containing proflavine at various hydrogen-ion concentrations were plotted. At all hydrogen-ion concentrations they were found to be of the same general shape and resembled those previously determined (Davies, Hinshelwood, and Pryce, *loc. cit.*). A typical set for proflavine is shown in Fig. 2, the family of curves extending to higher concentrations as the pH of the medium decreases.

From these and similar curves, the concentrations ($m_{\Delta L}$) required to produce a given value of ΔL at each pH were read off. Since proflavine is 100% ionised over the pH range employed, these concentrations were equal to those of the cations.

FIG. 3.

Relationship between log ($m_{\Delta L}$) *and pH for proflavine at different values of* ΔL.



$m_{\Delta L}$ *is the concentration of proflavine required to increase lag by* ΔL *minutes.*

Log ($m_{\Delta L}$) plotted against pH gives a series of parallel straight lines for the various assigned values of ΔL (Fig. 3). The following relationship is therefore obeyed:

$$\log (m_{\Delta L}) = n \log [H^+] + \log A$$

or,

$$\frac{m_{\Delta L}}{[H^+]^n} = A \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where A is a constant depending only on the particular value of ΔL chosen and n is the slope of the line. For normal cells in proflavine, $n = 0{\cdot}52$.

Similar experiments were made with 5-aminoacridine instead of proflavine, and curves of the same form were obtained, the value of the slope (n) being 0·88 (cf. the value of 0·89 found by Albert for 5-aminoacridine and *Bact. coli*).

Influence of pH on the Lag of Trained Cells in the Presence of Proflavine.—Bact. lactis aerogenes was allowed to become completely trained at a given concentration ($\overline{m}$) of proflavine and at a pH of 7·12 (10 to 15 sub-cultures were usually sufficient for this purpose). A culture of these trained cells was then used as the parent for lag–concentration determinations in various buffers, in exactly the same way as before, and the quantity ΔL again plotted against the concentration for each buffer. The family of curves was similar to that obtained with normal cells, but displaced towards higher proflavine concentrations. A typical set is shown in Fig. 4. As before, $m_{\Delta L}$ was read from the curves, and the logarithm plotted against the corresponding pH was found for each strain of cells to give a straight line. Fig. 5 shows these lines for $\Delta L = 1000$ minutes, and Table I gives the slopes of these lines together with the corresponding concentrations ($\overline{m}$) to which the cells were trained.

TABLE I.

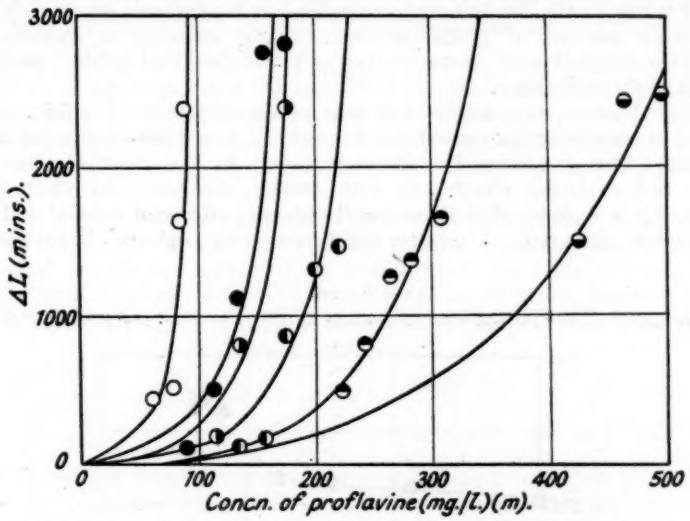
$\overline{m}$	0	21	44	71	90	109	115	118
Slope (n)	0·52	0·54	0·56	0·57	0·59	0·82	0·86	0·84

The table shows that the numerical value of the slope remains constant, within the experimental error, at 0·5—0·6 until $\overline{m}$ reaches about 100 mg./l. After this it increases to

0·8—0·9. In Fig. 6 the slope is plotted against the training concentration (m). Training to higher values of $\overline{m}$ was not carried out, since this would have necessitated high test concentrations of proflavine sulphate. Under these conditions the buffers would fail to keep the pH's near to the desired values.

FIG. 4.

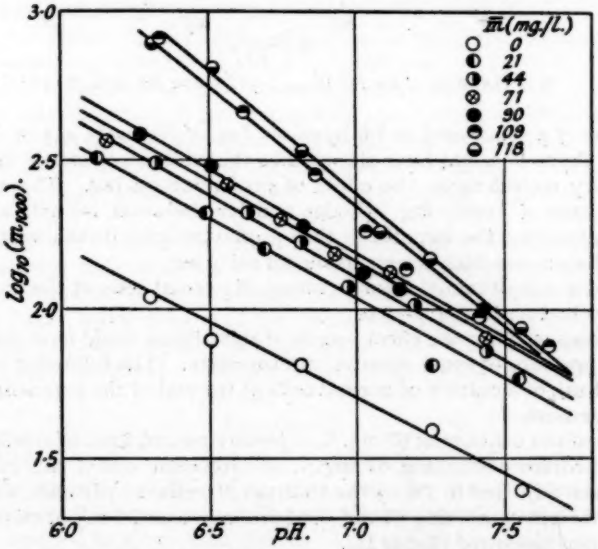
Lag–concentration curves for trained cells ($\overline{m} = 71$) *in proflavine at varying pH.*



pH : ○ 7·44, ● 7·12, ◑ 6·93, ◐ 6·74(5), ◓ 6·56, ◒ 6·14(5).

FIG. 5.

Relationship between log (m_{1000}) *and pH for cells trained to various proflavine concentrations* (m).



m_{1000} *is the concentration of proflavine required to increase lag by* 1000 *minutes.*

Influence of Neutral-salt Concentration.—It is well known that increase in the neutral salt concentration increases the uptake of cationic dyes by various fibres (Standing, *Trans. Faraday Soc.*, 1945, **41**, 410). The alkaline buffers used in these experiments contained a somewhat greater concentration of neutral sodium salt than the acid ones, since more sodium hydroxide solution was added to the potassium dihydrogen phosphate solution in their preparation. The

increased activity of the acridines in alkaline solution might thus have been due to their greater absorption by the cell at this greater neutral salt concentration. The importance of this factor had to be determined in special experiments.

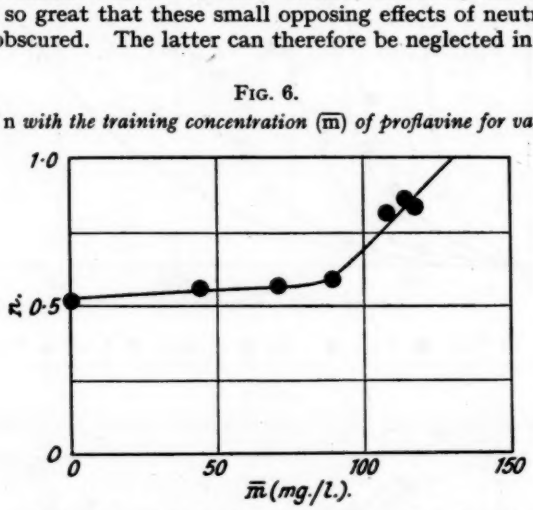
The concentration of neutral salt was calculated from the volume of the 4N-sodium hydroxide added to the phosphate solution. The buffer of pH = 7·55 contained, for example, 0·011 equiv. per l. of " neutral " sodium salt more than the normal buffer.

The lags of normal cells (transferred at the end of logarithmic growth) in normal media containing a fixed amount of proflavine and varying amounts of sodium chloride were determined. Two controls with, respectively, no proflavine, and neither proflavine nor salt, were inoculated at the same time.

The results of these experiments showed that in actual fact 0·011 equiv. per l. of sodium chloride instead of increasing lag caused decreases of 7% in a 1000-minute lag and of 27% in a 4000-minute lag. This concentration of neutral salt in the most alkaline buffers might therefore have had a similar effect. In such buffers, however, the effect of proflavine in lengthening the lag is so great that these small opposing effects of neutral salt concentrations would be completely obscured. The latter can therefore be neglected in the future discussion.

FIG. 6.

Variation of n *with the training concentration* $(\overline{m})$ *of proflavine for various strains.*



n *is the slope of the log* (m_{1000})*–pH curve for each strain.*

Effect of Filtrate of pH adjusted to 7·0 *upon the Lag of Normal Cells in Proflavine.*—Davies, Hinshelwood, and Pryce (*loc. cit.*) have shown that the addition of sterile filtrate from a normal culture reduces very considerably the effect of proflavine on lag. The effect is particularly pronounced in the case of " early lag " (Lodge and Hinshelwood, *loc. cit.*) and the phenomenon was regarded as supporting the hypothesis that proflavine inhibits the synthesis of an essential intermediate the absence of which is responsible for early lag.

The sterile filtrate was obtained from a culture of normal cells at the end of the logarithmic phase and therefore had a pH of 4·5—5·0.

The question arises whether the effectiveness of this filtrate could have been due to its acidity rather than to the presence of some essential intermediate. The following media were therefore inoculated with 0·1 ml. of a culture of normal cells at the end of the logarithmic phase :

(*a*) A normal medium.

(*b*) A normal medium containing 67 mg./l. of proflavine and 2 ml. of sterile water.

(*c*) A normal medium containing 67 mg./l. of proflavine and 2 ml. of sterile filtrate, the pH of which had been adjusted to 7·0 by the addition of sodium hydroxide solution.

(*d*) A normal medium containing 67 mg./l. of proflavine and 2 ml. of sterile filtrate of pH 5·0.

The lags were then measured (Table II).

TABLE II.

Medium	(*a*)	(*b*)	(*c*)	(*d*)
Proflavine concn. (mg./l.)	0	67	67	67
Lag (L), mins.	0	920	320	130
	($=L_0$)	940	350	140
Average ΔL ($L - L_0$), mins.	0	930	335	135

Table II shows that both filtrates reduced the lag considerably, that of pH 5·0 being more effective than that of pH 7·0. The adjusted filtrate causes a lag reduction of about 600 minutes, and the acid filtrate a further reduction of 200 minutes. The effect of the unadjusted filtrate cannot therefore be due mainly to its acidity, and the presence in filtrate of some essential intermediate can still be inferred. Nevertheless, the total reduction by filtrate of the lag in proflavine is due to :

(i) the presence of an effective concentration of some essential intermediate, and
(ii) the acidity of the filtrate.

Behaviour of Cells repeatedly Sub-cultured in Acid Media.—On repeated sub-culture of bacteria in an acid medium, the cells might conceivably adapt themselves to resist the effects of the acid—for example, by reducing the number of negative groups which attract hydrogen ions. Such a response would probably affect the sensitivity of the bacteria to the action of the proflavine cation.

In order to study this question, a strain of *Bact. lactis aerogenes* which had been sub-cultured many times in the usual medium of pH 7·1—7·2 was transferred to a similar medium of pH 6·17. The total bacterial population supported by this medium was much lower, as previously observed (Lodge and Hinshelwood, *J.*, 1939, 1683), and did not increase with repeated sub-culture (Table III).

TABLE III.

No. of sub-cultures in medium of pH 6·17	1	5	11	12	17	20	21	24	26	28	32	33	34
Stationary population (no./ml. ÷ 1·25 × 10^6)	410	510	580	520	450	400	480	450	630	460	400	470	530

After the 23rd culture in acid medium, lag–concentration curves in proflavine at various pH's were determined in exactly the same way as before. The curves were of the usual shape and the same effect of hydrogen ions on the activity of the proflavine was displayed. The slope of the log ($m_{\Delta L}$)–pH plot was 0·49, which, within the experimental error, is the same as for normal cells.

Lag–concentration curves at two of the pH's used in the test were determined for the parent strain of normal cells. Within the experimental error, these coincided with the corresponding curves for the cells sub-cultured in the acid medium.

It was therefore concluded that repeated sub-culture in more acidic media did not affect the groups and enzyme systems involved in the action of proflavine.

Behaviour of Cells Trained to Proflavine in an Acid Medium.—A training experiment somewhat similar to that described in the last section was performed. Cells were successively sub-cultured in an acidic phosphate buffer (pH 6·3) and 157 mg./l. of proflavine. This concentration at this pH at first caused a lag of about 3000 minutes which decreased to zero after 20 sub-cultures. The inhibitory action is here much less than it would be at the normal pH.

Lag–concentration curves in proflavine at pH 6·3 and 7·12 were then determined. In this way the effective training concentration at pH 7·12 corresponding to the actual training at pH 6·3 and 157 mg./l. could be found. In the test at the lower hydrogen-ion concentration the displacement of the curve away from that for the untrained cells corresponded to a training concentration (at that pH) very much less than 157 mg./l. Thus the response to training is determined by the actual degree of inhibition rather than by the concentration of the toxic agent.

DISCUSSION.

(1) *The Relationship between* $m_{\Delta L}$ *and the Hydrogen-ion Concentration.*—In equation (1) which applies to the growth of *Bact. lactis aerogenes* in the presence of proflavine and 5-aminoacridine : $n = ca.$ 0·5 for normal cells and cells trained to proflavine up to 100 mg./l., $n = ca.$ 0·8 for cells trained to more than 100 mg./l. of proflavine, and $n = ca.$ 0·9 for normal cells, tested in 5-aminoacridine.

Mention has already been made of the suggestion that the acridinium ions and hydrogen ions are in competition for similar negative sites on the surface of an enzyme whose functioning is inhibited by the acridine. The competition of three molecular species—substrate molecules (S), acridinium ions (D^+), and hydrogen ions (H^+)—has in general to be considered. The possibilities include :

(*a*) Competition on the surface between acridine cations and hydrogen ions only. This

involves the assumption that the adsorption of hydrogen ions on the protein, unlike that of the drug cations, does not interfere with the utilisation of the substrate molecules by the enzyme. This is not unlikely, since in its normal active condition the protein of an enzyme is continually exchanging hydrogen ions with its surrounding medium.

In the following treatment the reactions between the negative groups on the protein (enzyme) and the two types of cation are regarded as independent. Let these negative groups be represented by P^-, and the drug cations by D^+. m is the total concentration of the acridine in solution (100% ionisation). Then,

$$PH = H^+ + P^-$$
$$PD = D^+ + P^-$$

Hence, $$K_1 = \frac{[H^+][P^-]}{[PH]}, \text{ and } K_2 = \frac{m\,[P^-]}{[PD]}$$

$$\therefore \quad \frac{K_1}{K_2} = \frac{[H^+][PD]}{[PH]\,m}$$

If it is assumed that for a given value of the lag the ratio $\frac{[PD]}{[PH]}$ is constant, then m becomes $m_{\Delta L}$ and

$$\frac{m_{\Delta L}}{[H^+]} = \text{constant} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

According to this, the value of the index n of equation (1) should be unity and should be the same for all compounds competing with hydrogen ions. Since neither of these statements is true for *Bact. lactis aerogenes* in the presence of proflavine and 5-aminoacridine, this simple statement is inadequate. This is not unexpected, since various doubtful assumptions are involved, for example, that, in spite of the difference in their size, shape, and charge distribution, the activity coefficients of the hydrogen and acridinium ions are the same. Inaccuracies of this sort may be quite sufficient in themselves to account for the departure of the value of n from unity, but are difficult to estimate in any quantitative way.

(*b*) Competition involving substrate molecules. Substrate molecules (S) may also be involved in equilibria with hydrogen ions and even drug cations (D^+), giving SH^+ and SD^+, respectively. The cations SH^+ may then compete on the surface with D^+, H^+, and SD^+, it being assumed that SH^+ is the only form of the substrate utilisable by the enzyme. Increasing hydrogen-ion concentration would then increase the concentration of SH^+ in the cell and hence its adsorption relative to other ions. Qualitatively this would account for the observed antagonism of proflavine and hydrogen ions.

The competition of SH^+, SD^+, D^+ and H^+ for a limited number of adsorption points on an enzyme suface can be treated by the usual elementary methods. If, for any given strain of cells, S is taken to be constant, and if it is assumed that, for any assigned value of ΔL, the fraction of surface occupied by SH^+ is constant, then, replacing D^+ by $m_{\Delta L}$, we obtain

$$\frac{A[H^+]}{1 + B[H^+] + Cm_{\Delta L}} = \text{constant} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where, for a given value of ΔL, A, B, C are constants.

This equation, which is a Langmuir equation for adsorption of several types of molecules on a limited number of sites, is not of the form of equation (1). Over a limited range of hydrogen-ion concentration, however, it might approximate to an expression of the observed type.

(2) *Modification of the* ΔL–m *Relationship to include the pH Effect.*—On the basis of their theory of adaptation to proflavine (and other drugs), Pryce and Hinshelwood (*Trans. Faraday Soc.*, 1947, in the press) derived an expression of the following form for the dependence of the lag (L) of trained and untrained cells on the concentration (m),

$$L = \frac{\text{constant}}{f(S_0) + \phi(\bar{m}) - \phi(m)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$\phi(m)$ is a function of the test concentration (for proflavine a simple linear one) and $\phi(m)$ the same function of the concentration $(\bar{m})$ to which the strain has become adapted. This equation,

however, was only intended to apply to cultures at a constant pH. As a suitable modification which allows for the influence of pH the following is suggested.

$$L = \frac{\text{constant}}{f(S_0) + \left[\frac{\phi(\bar{m})}{[\bar{H}^+]^n}\right] - \left[\frac{\phi(m)}{[H^+]^n}\right]} \quad \ldots\ldots\ldots \quad (5)$$

where $[H^+]$ = test concentration, and $[\bar{H}^+]$ = training concentration of hydrogen ion.

No change is made in the term $f(S_0)$ since the lag in the absence of the acridine is independent of the pH. For any particular strain of cells, trained or untrained, $f(S_0)$, $\phi(\bar{m})$, $[\bar{H}^+]$ are all constant. Hence $\frac{[H^+]^n}{\phi(m_{\Delta L})}$ is constant for a given strain of cells at a constant value of L (and therefore of ΔL).

For proflavine, $\phi(m) \propto m$ (Pryce and Hinshelwood, *loc. cit.*) and therefore,

$$\frac{m_{\Delta L}}{[H^+]^n} = \text{constant}$$

which is equation (1). Equation (5), therefore, adequately expresses the experimental facts. It also predicts correctly the behaviour on training to proflavine at various hydrogen-ion concentrations.

When the lag approaches infinity, the denominator of equation (5) becomes zero.

$$f(S_0) + \frac{\phi(\bar{m})}{[\bar{H}^+]^n} = \frac{\phi(m)}{[H^+]^n}$$

For proflavine, $\phi(m) = f'\,m$ (where f' is a constant)

$$m = \left(\frac{[H^+]}{[\bar{H}^+]}\right)^n + \frac{f(S_0)}{f'}[H^+]^n$$

$\frac{f(S_0)}{f'}$ = constant, if all the trained strains are derived from the same parent strain.

Hence, if the cells are trained to concentration $\bar{m}$ of proflavine at a hydrogen-ion concentration $[\bar{H}^+]$, and tested in the presence of the acridine at a lower hydrogen-ion concentration, $[H^+]$, the m value observed will correspond to that obtained by training at a lower value of $\bar{m}$ at the lower hydrogen-ion concentration, $[H^+]$. This is the result described in the experimental section.

No theoretical reason for the substitutions leading from equation (4) to equation (5) is advanced at present, but the latter expresses observed results correctly.

(3) *The Effect of Training on the Relation between* $m_{\Delta L}$ *and* (H^+).—In equation (1), the index n remains constant at about 0·5 with proflavine for strains trained up to about 100 mg./l. Above this limit n increases to about 0·9.

The experiments with trained cells were made because it was considered that any change in this index on training would indicate that the competitive powers of the hydrogen ion relative to acridinium had altered and that qualitative modifications of the enzyme surface had occurred.

The results suggest that such modifications occur only when training is carried to concentrations of proflavine greater than about 100 mg./l.

The introduction of some new factor in the training process at about this stage is suggested by two other pieces of evidence :

(i) The attainment of a given degree and stability of training to concentrations of proflavine greater than 100 mg./l. requires a greater number of subcultures than does training to lower concentrations. The stabilisation of complex qualitative changes in the enzymes as part of the adaptive process might be expected to require a longer time than simple quantitative expansion, which has been shown to occur after two or three divisions in the presence of the acridine (Davies, Hinshelwood, and Pryce, *loc. cit.*). The change in the value of n and the increase in the difficulty of training at roughly the same concentration are probably not unconnected.

(ii) Expressions of the same type as equation (4), based on a theory of quantitative enzyme expansion during adaptation, have been shown to predict the general form of a family of lag–concentration curves for strains trained to increasing concentrations of proflavine. Satisfactory quantitative agreement is also obtained for strains trained up to about 95 mg./l., one set of constants in the equation predicting the shape and position of the curves for the

various trained strains (Davies, Hinshelwood, and Pryce, *Trans. Faraday Soc.*, 1945, **41**, 465). Above the limit of about 95 mg./l., however, divergences begin to occur and certain relationships between the constants, which up to this point have been obeyed, begin to break down. This is evidence of an indirect kind only, but it is of interest that the divergences declare themselves at roughly the same point as that where training becomes more difficult and the value of n begins to increase.

Each of the above facts by itself is probably inadequate to justify any particular conclusion, but, taken together, they constitute evidence that adaptation to concentrations of proflavine of up to 100 mg./l. consists of quantitative enzyme expansions only, and that above this limit qualitative modifications of enzymes may also be involved.

Evidence of enzyme modifications more complex and far-reaching than a simple quantitative expansion is also afforded by cells trained to concentrations of proflavine of the order of 1500 mg./l. (*idem, ibid.*, p. 777) and depends upon the unusual shape of the lag–concentration curves given by these strains after passage through normal media. The concentrations involved are very much greater than those in the present experiments and the interpretation of the result may be different, but the effect in question does provide evidence of complex changes which may accompany training to concentrations of proflavine at the other end of the concentration scale.

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245. *Melanin and its Precursors. Part I. The Synthesis of 5 : 6 : 5′ : 6′-Tetrahydroxyindigo.*

By JOHN HARLEY-MASON.

As previously reported briefly (*Nature*, 1947, **159**, 338) *5 : 6 : 5′ : 6′-tetrahydroxyindigo* has been synthesised by the action of acetone and alkali on *2-nitro-4 : 5-bis(methoxymethoxy)-benzaldehyde* and subsequent acid hydrolysis of the *5 : 6 : 5′ : 6′-tetra(methoxymethoxy)indigo* thus obtained. Its properties are very unlike those of melanin, and it is further shown that melanin is unlikely to contain a quinone or semiquinone derived from it.

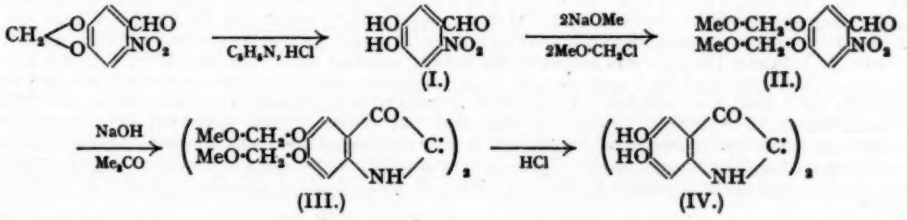
IT has recently been suggested (Clemo and Weiss, *J.*, 1945, 702) that 5 : 6 : 5′ : 6′-tetrahydroxyindigo (IV) or a quinone or semiquinone derived from it by oxidation is an important constituent of melanin, the black pigment produced by the oxidation of tyrosine and 3 : 4-dihydroxyphenylalanine. It was of interest, therefore, to synthesise the tetrahydroxyindigo by an unambiguous route and to compare its properties with those of melanin.

Three earlier attempts to obtain this compound have been recorded. (1) Hayduck (*Ber.*, 1903, **36**, 2930) unsuccessfully attempted to demethylate a compound which he considered to be 5 : 6 : 5′ : 6′-tetramethoxyindigo; however, he showed later (*Ber.*, 1903, **36**, 3528) that his starting material was in fact the 6 : 7 : 6′ : 7′-isomer. (2) Clemo and Weiss (*loc. cit.*) attempted to demethylenate 5 : 6 : 5′ : 6′-bismethylenedioxyindigo (piperonal indigo) under various conditions, but obtained only alkali-insoluble products giving unsatisfactory analytical figures. (3) Parijs (*Rec. Trav. chim.*, 1930, **49**, 18) claimed that the compound can be obtained by the action of acetone and alkali on 6-nitroprotocatechuic aldehyde (I); he stated that "The addition of alkali to an acetone solution [of 6-nitroprotocatechuic aldehyde] afforded a solution of the corresponding indigo: subsequent addition of acid precipitated dark blue 5 : 6 : 5′ : 6′-tetrahydroxyindigo". This claim could not be substantiated by the author, only the starting materials being recovered after the treatment described. Indeed, the failure is hardly surprising in view of the fact that Friedländer and Schenk (*Ber.*, 1914, **47**, 3040) found that 2-nitro-5-hydroxybenzaldehyde failed altogether to condense with acetone and alkali.

The most promising approach to the tetrahydroxyindigo appeared to be the condensation of a derivative of 6-nitroprotocatechuic aldehyde, in which the hydroxyl groups were suitably protected, with acetone and alkali, and subsequent removal of the protecting groups. Since Friedländer and Schenk (*loc. cit.*) had observed that the tosyl derivative of 2-nitro-5-hydroxybenzaldehyde could be converted into the corresponding indigo with acetone and alkali, and that the tosyl groups could be removed subsequently, it seemed probable that a similar procedure would be applicable to 6-nitroprotocatechuic aldehyde. This compound was first prepared by Parijs (*loc. cit.*) by the action of acetyl nitrate on 6-nitropiperonal diacetate and subsequent

hydrolysis. On repetition of this curious reaction, which appears to involve nitration of the methylene group, it was found that the yields obtained were very much lower than the 95% claimed by Parijs, and that even smaller yields were obtained in larger-scale runs. Accordingly, other methods of demethylenation of 6-nitropiperonal were examined. The compound proved remarkably resistant, and no satisfactory result could be achieved by heating with sulphuric acid of various concentrations, heating with hydrobromic acid in acetic acid to 150°, or by treatment with phosphorus pentachloride. Eventually it was found that the methylene group could be removed by heating with pyridine hydrochloride at 160—170°. This reaction is, of course, closely analogous to the demethylations using pyridine hydrochloride recorded by Prey (*Ber.*, **1942**, **75**, 350) and appears to be the first case of the use of this reagent for demethylenation, as distinct from demethylation. It was found to be essential that the pyridine hydrochloride be as dry as possible or the yield was much reduced, presumably owing to the formation of formaldehyde which then condensed with the hydroxy-aldehyde. The reaction is also strongly exothermic and a considerable excess of pyridine hydrochloride must be used to prevent over-heating.

Treatment of 6-nitroprotocatechuic aldehyde with benzenesulphonyl chloride and alkali gave the *bisbenzenesulphonyl* derivative, which surprisingly, however, failed to react at all with acetone and alkali. An alternative protecting group was therefore sought. When the disodium salt of the dihydroxy-aldehyde was treated with two molecules of chloromethyl ether in benzene, *2-nitro-4* : *5-bis(methoxymethoxy)benzaldehyde* (II) was obtained, and from it, 5 : 6 : 5′ : 6′-*tetra(methoxymethoxy)indigo* (III) in good yield by the action of acetone and alkali. The latter compound could be recrystallised unchanged from glacial acetic acid, but on addition of water and a trace of hydrochloric acid to the acetic acid solution, hydrolysis occurred, giving the required tetrahydroxyindigo. As it could not be recrystallised, the compound was purified by dissolving it in dilute aqueous alkali, filtering the intense permanganate-violet solution in the absence of air, and immediately precipitating the indigo by addition of acetic acid. Thus prepared, it formed a greenish-black powder which retained a molecule of water of crystallisation even after drying in a high vacuum. It was practically insoluble in the usual organic solvents, but was slightly soluble in pyridine, giving a blue solution. The identity of the product was confirmed (*a*) by acetylation with acetic anhydride in pyridine which gave a *tetra-acetyl* derivative which could be recrystallised from nitrobenzene, and (*b*) by methylation with methyl sulphate and alkali, giving 5 : 6 : 5′ : 6′-*tetramethoxyindigo*, identical with that obtained from the treatment of 6-nitroveratraldehyde with acetone and alkali.



On reduction with sodium hydrosulphite (dithionite) and alkali, the tetrahydroxyindigo gave a deep yellow vat, which reoxidised in air to give an intense violet solution, which however soon faded to brown with further absorption of oxygen.

The behaviour of melanin towards sodium hydrosulphite and alkali has been described by Figge (*Proc. Soc. Biol. Exp. Med.*, **1939**, **41**, 127). Reduction was found to occur, giving a brownish leuco-compound which could be re-oxidised to melanin by potassium ferricyanide. This observation has been confirmed in the present work, and in addition it was found that the leuco-compound, like melanin itself, is insoluble in aqueous alkali. Tetrahydroxyindigo is thus quite different from melanin. Furthermore, a quinone or semiquinone derived by oxidation of the tetrahydroxy-compound would undoubtedly give on reduction with sodium hydrosulphite the same leuco-compound as that obtained from (IV) itself.

In the preparation of the aldehyde (II) a by-product was obtained which was soluble in alkali. Analysis indicated that this was a monomethoxymethyl derivative of 6-nitroprotocatechuic aldehyde. To determine the location of the methoxymethyl group, the compound was methylated with methyl sulphate and alkali, and then hydrolysed with acid. The product was 6-nitro*iso*vanillin (Pschorr and Stoehrer, *Ber.*, **1902**, **35**, 4394), so the original compound was *2-nitro-4-hydroxy-5-methoxymethoxybenzaldehyde*.

Experimental.

6-*Nitroprotocatechuic Aldehyde*.—6-Nitropiperonal (25 g.) and anhydrous pyridine hydrochloride (50 g.) were mixed and heated to 150° in an oil-bath. Commencement of the exothermic reaction was indicated by the appearance of bubbles in the liquid, and the temperature was kept below 170° by removing the flask from the oil-bath when necessary. When the reaction had subsided, the mixture was heated at 170° for 0·5 hour. After cooling the mass was boiled with water (300 c.c.) with the addition of charcoal, cooled, and filtered through charcoal to remove tar. Concentrated hydrochloric acid (10 c.c.) was added to the filtrate which was then extracted with ether in a continuous extractor. Evaporation of the ether yielded crude 6-nitroprotocatechuic aldehyde which was purified by recrystallisation from water; 9—12 g., m. p. 203°.

***Bisbenzenesulphonyl derivative*.** 6-Nitroprotocatechuic aldehyde (2 g.) was dissolved in 10% aqueous sodium hydroxide (30 c.c.) and shaken with benzenesulphonyl chloride (4 c.c.). When the reaction was complete, the solid *product* was collected and recrystallised from benzene–light petroleum; m. p. 159° (Found: C, 49·5; H, 3·0. $C_{19}H_{13}O_8NS$ requires C, 49·3; H, 2·8%).

2-*Nitro*-4 : 5-*bis*(*methoxymethoxy*)*benzaldehyde* (II).—6-Nitroprotocatechuic aldehyde (5·5 g.) was dissolved in methanol (30 c.c.) and a solution of sodium methoxide prepared by dissolving sodium (1·4 g.) in methanol (30 c.c.) was added. The solvent was distilled off in a vacuum under nitrogen, leaving the disodium salt as a deep violet cake. Dry benzene (60 c.c.) was added, and the cake was broken up in order to obtain a fine suspension of the salt. A solution of pure redistilled chlorodimethyl ether (4·9 g.) in dry benzene (15 c.c.) was next added. Reaction proceeded, and the mixture became warm. After an hour's heating under reflux on the water-bath, the mixture was cooled and filtered from the separated sodium chloride. The filtrate was extracted with 2% sodium hydroxide solution until the aqueous layer was only faintly yellow, the benzene solution separated, dried (Na_2SO_4), and distilled down to 10 c.c. Light petroleum (b. p. 60—80°) (20 c.c.) was added to the hot solution. After standing overnight in the ice-chest, the 2-*nitro*-4 : 5-*bis*(*methoxymethoxy*)*benzaldehyde* which had separated was collected and recrystallised from benzene–petrol. It formed pale yellow prisms, m. p. 100—101° (Found: C, 48·5; H, 4·7. $C_{11}H_{13}O_7N$ requires C, 48·8; H, 4·8%).

The alkaline extract was acidified with acetic acid, and after standing overnight at 0°, the precipitate was collected and recrystallised from dilute methanol; pale yellow needles, m. p. 110°, were obtained (Found: C, 47·2; H, 4·3. $C_9H_9O_6N$ requires C, 47·7; H, 4·0%). That this compound was 2-*nitro*-4-*hydroxy*-5-*methoxymethoxybenzaldehyde* was proved as follows. The compound (1 g.) was dissolved in 5% sodium hydroxide solution (10 c.c.), and methyl sulphate (1 c.c.) added. After being heated at 80° with occasional shaking for 0·5 hour, the mixture was cooled and the solid product filtered off. It was hydrolysed by boiling for 1 hour with dilute hydrochloric acid, and the 6-nitro*iso*vanillin thus obtained was collected and recrystallised from dilute alcohol; m. p. and mixed m. p. 187° (Pschorr and Stoehrer, *loc. cit.*, give m. p. 189°).

5 : 6 : 5′ : 6′-*Tetra*(*methoxymethoxy*)*indigo* (III).—2-Nitro-4 : 5-bis(methoxymethoxy)benzaldehyde (2 g.) was dissolved in acetone (8 c.c.), and 10% sodium hydroxide solution (1 c.c.) added. After an hour, water (30 c.c.) and 10% sodium hydroxide solution (5 c.c.) were added with stirring. Next day the precipitated *indigo* was collected and washed with water and alcohol. On recrystallisation from glacial acetic acid it formed deep green needles, containing a molecule of acetic acid, which was lost on drying at 110° in a vacuum (Found: C, 55·7; H, 4·8. $C_{24}H_{28}O_{10}N_2,CH_3{\cdot}CO_2H$ requires C, 55·6; H, 5·3%. Found, in material dried at 110°: C, 56·7; H, 5·4. $C_{24}H_{28}O_{10}N_2$ requires C, 57·2; H, 5·2%).

5 : 6 : 5′ : 6′-*Tetrahydroxyindigo* (IV).—The indigo (III) (1·5 g.) was dissolved in boiling glacial acetic acid (40 c.c.). Water (50 c.c.) was added to the boiling solution with vigorous stirring, giving a greenish suspension. Concentrated hydrochloric acid (1 c.c.) was next added, and the mixture heated on a boiling water-bath for 1 hour with stirring, the greenish tetramethoxymethoxy-compound being converted into the black tetrahydroxyindigo. After cooling, this was collected and dissolved in cold 1% sodium hydroxide solution under nitrogen, rapidly filtered from impurities, and the intense violet filtrate at once acidified with dilute acetic acid. The black precipitate was collected and thoroughly washed with water, alcohol, and ether (Found: C, 55·9; H, 3·6; N, 8·1. $C_{16}H_{10}O_6N_2,H_2O$ requires C, 56·0; H, 3·5; N, 8·15%).

5 : 6 : 5′ : 6′-*Tetra-acetoxyindigo*.—The *tetrahydroxyindigo* (0·2 g.) was suspended in pyridine (5 c.c.), and acetic anhydride (5 c.c.) added. The mixture was heated for 0·5 hour at 100° with stirring. After cooling, the *product* which had separated was filtered off and recrystallised from nitrobenzene in which it was sparingly soluble. It formed small dark blue prisms with an intense coppery lustre and decomposed without melting (Found: C, 58·0; H, 3·8; N, 5·5. $C_{24}H_{18}O_{10}N_2$ requires C, 58·3; H, 3·65; N, 5·7%).

5 : 6 : 5′ : 6′-*Tetramethoxyindigo*.—(a) *From* **6-*nitroveratraldehyde*.** 6-Nitroveratraldehyde (2 g.) was dissolved in acetone (5 c.c.) and 5 drops of 10% sodium hydroxide solution added. After an hour, water (30 c.c.) and more 10% alkali (5 c.c.) were added, and after standing overnight the dark greenish precipitate of the *indigo* was collected and extracted with boiling alcohol. It was then recrystallised from nitrobenzene; yield 0·4 g. (The yield was not improved by heating the reaction mixture.) (Found: C, 62·5; H, 4·8. $C_{20}H_{18}O_6N_2$ requires C, 63·0; H, 4·7%). On cooling, the alcoholic extract deposited yellow plates. These were collected and recrystallised from alcohol (charcoal); m. p. 172°. Analysis indicated that this compound was 2-*nitro*-4 : 5-*dimethoxybenzylideneacetone* (Found: C, 56·7; H, 5·2. $C_{12}H_{13}O_5N$ requires C, 57·1; H, 5·2%).

(b) *From* (IV). The tetrahydroxyindigo (0·4 g.) was dissolved in 5% sodium hydroxide solution (5 c.c.) under nitrogen, and methyl sulphate (0·8 c.c.) added. The mixture was heated at 80° for 1 hour with frequent shaking, air being excluded. After cooling, the tetramethoxyindigo was collected, washed with alcohol, and recrystallised from nitrobenzene.

That the products from (a) and (b) were identical was shown by the fact that both could be oxidised to the same product, namely, 5 : 6-dimethoxyisatin.

The indigo (0·2 g.) was stirred with nitric acid (*d* 1·3) (1·5 c.c.) and warmed on the water-bath for a

few minutes until reaction was complete. After cooling, the product was filtered off, extracted with dilute sodium hydroxide, filtered from impurities, and the isatin precipitated from the filtrate by the addition of hydrochloric acid; yellow-orange prisms from ethyl acetate, m. p. 250—253° with preliminary darkening (Found: C, 56·3; H, 4·6. Calc. for $C_{10}H_9O_4N$: C, 56·6; H, 4·35%). Hahn and Tulus (*Ber.*, 1941, **74**, 518) give m. p. 250—252°.

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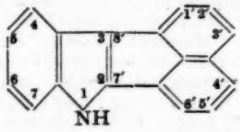
246. *The Action of Nitric Acid on Polycyclic Indole Derivatives. Part XIV. Acenaphthyleno(7′ : 8′ : 2 : 3)indole.*

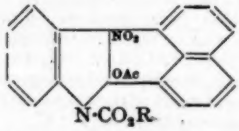
By B. BANNISTER and S. G. P. PLANT.

The double bond in the 2 : 3-position in the 1-acylacenaphthyleno(7′ : 8′ : 2 : 3)indoles has been shown to be relatively reactive so that nitration in acetic acid has given not only the 6(or 4)-nitro-derivatives, but also, in some cases, compounds formed by the addition of NO_2 and OAc.

PRODUCTS formed by the addition of 2OH, NO_2 and OH, or NO_2 and OAc, at the 2 : 3-position frequently accompany the nitro-derivatives prepared by the nitration of certain *N*-acylindoles in acetic acid, but the feature is generally less well developed in the more complex polycyclic indoles than in the simpler substances such as 2 : 3-dimethylindole and tetrahydrocarbazole (*J.*, 1928, 1840; 1931, 93; 1932, 2192; 1933, 955). Fennell and Plant (*J.*, 1932, 2872) found that nitration of 1-acyl-2 : 3-diphenylindoles resulted in formation of additive compounds containing the phenanthracarbazole skeleton, and the observations suggest that the addition reactions are more likely to proceed with substances in which the indole structure is fused at the 2 : 3-position to an active double bond in another polynuclear system. In this connection, interest attaches to acenaphthyleno(7′ : 8′ : 2 : 3)indole (I), which has been prepared by Sircar and Gopalan (*J. Indian Chem. Soc.*, 1932, **9**, 297; see also Korczynski, Brydowna, and Kierzek, *Gazzetta*, 1926, **56**, 903) by applying the Fischer reaction to acenaphthenone phenylhydrazone.

(I.)





(II.)

The action of one molecular proportion of nitric acid on its 1-*acetyl*, 1-*benzoyl*, 1-*carbomethoxy*-, 1-*carbethoxy*-, and 1-*cinnamoyl* derivatives in hot acetic acid has given in each case a simple nitro-compound. There is little doubt that the substituent in these substances occupies the 6-position, which is the one almost invariably taken in analogous cases. This has been partly proved by the synthesis of one of them by converting *acenaphthenone* m-*nitrophenylhydrazone* into the corresponding indole and cinnamoylating the product, and by converting three of the others into the same cinnamoyl derivative by hydrolysis and subsequent acylation, results which leave only the 4-position as a possible alternative.

Furthermore, the 1-carbomethoxy- and the 1-carbethoxy-derivative have given substantial quantities of the addition compounds, *methyl* and *ethyl* 3-*nitro*-2-*acetoxy*-2 : 3-*dihydroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate* (II; R = Me or Et), which are obviously analogous to the nitroacetoxy-compounds already described (*J.*, 1931, 1990; 1938, 1214; 1939, 1534) and indicative of the reactive character of the double bond. The 1-cinnamoyl derivative has also given a colourless *substance*, evidently additive in type, the structure of which is less obvious. Its molecular formula appears to be $C_{29}H_{20}O_6N_2$, which suggests the addition of OH and OAc at the double bond together with a nitro-group as a substituent.

In studying other applications of the Fischer reaction to hydrazones of acenaphthenone, it was noted that the process went normally with the 2-*chloro*-5-*nitrophenylhydrazone* and the p-*bromophenylhydrazone*, but the corresponding indole was not obtained from the o-*nitrophenylhydrazone*.

EXPERIMENTAL.

1-*Acyl- and* 1-*Alkyl-acenaphthyleno*(7′ : 8′ : 2 : 3)*indoles*.—Acetyl chloride (1·8 c.c.) was gradually added, with shaking, to a solution of acenaphthyleno(7′ : 8′ : 2 : 3)indole (1 g., m. p. 235°, prepared by

the method of Sircar and Gopalan, *loc. cit.*) in acetone (20 c.c.) containing aqueous potassium hydroxide (3·4 g. of 66%). The addition of water precipitated 1-*acetylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*, which was obtained from *cyclo*hexanone in orange-red prisms (0·9 g.), m. p. 207° (Found : N, 4·8. $C_{20}H_{13}ON$ requires N, 4·9%).

In similar experiments using benzoyl chloride, methyl chloroformate, ethyl chloroformate, cinnamoyl chloride, methyl sulphate, or ethyl sulphate, instead of acetyl chloride, the following were respectively obtained in good yields : 1-*benzoylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*, dark red prisms, m. p. 148—149°, from acetic acid (Found : N, 4·0. $C_{25}H_{15}ON$ requires N, 4·1%); *methyl acenaphthyleno*(7′ : 8′ : 2 : 3)-*indole*-1-*carboxylate*, golden brown plates, m. p. 154°, from acetic acid (Found : N, 4·5. $C_{20}H_{13}O_2N$ requires N, 4·7%); *ethyl acenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate*, orange-red needles, m. p. 106°, from acetic acid (Found : N, 4·5. $C_{21}H_{15}O_2N$ requires N, 4·5%); 1-*cinnamoylacenaphthyleno*-(7′ : 8′ : 2 : 3)*indole*, orange-red plates, m. p. 166°, from acetic acid (Found : N, 3·5. $C_{27}H_{17}ON$ requires N, 3·8%); 1-methylacenaphthyleno(7′ : 8′ : 2 : 3)indole, scarlet plates, m. p. 204°, from acetic acid, identical (mixed m. p.) with the product obtained by applying the Fischer reaction to acenaphthenone phenylmethylhydrazone as described by Sircar and Gopalan (*loc. cit.*); and 1-*ethylacenaphthyleno*-(7′ : 8′ : 2 : 3)-*indole*, red plates. m. p. 182°, from acetic acid (Found : N, 5·3. $C_{20}H_{15}N$ requires N, 5·2%).

All the above acyl compounds were hydrolysed to acenaphthyleno(7′ : 8′ : 2 : 3)indole by refluxing their solutions in aqueous-alcoholic potassium hydroxide for ½ hour, the indole crystallising on cooling. Attempts to repeat the preparation of the benzoyl and the carbethoxy-derivative were not always successful for no apparent reason, the unchanged indole being recovered on several occasions. In this connection it is of interest that an attempt to prepare the 1-phenylacetyl derivative under similar conditions with phenylacetyl chloride failed.

Nitration of 1-*Acetyl- and* 1-*Benzoyl-acenaphthyleno*(7′ : 8′: 2 : 3)*indole*.—When a suspension of the acetyl compound (1 g.) in glacial acetic acid (50 c.c.) at 110° was treated with nitric acid (0·15 c.c., *d* 1·5), dissolved in acetic acid (1 c.c.), with stirring, the solid soon went into solution, and, on cooling, 6(or 4)-*nitro*-1-*acetylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole* (0·4 g.) began to separate. It was collected after 3 hours and recrystallised from acetic acid, from which it was obtained in orange-red needles, m. p. 275° (Found : N, 8·5. $C_{20}H_{12}O_3N_2$ requires N, 8·5%).

After a solution of the 1-benzoyl compound (1·5 g.) in glacial acetic acid (20 c.c.) at its boiling point had been similarly treated 6(or 4)-*nitro*-1-*benzoylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole* (1 g.), orange-red needles, m. p. 225° (decomp.), from acetic acid, was obtained (Found : N, 6·9. $C_{25}H_{14}O_3N_2$ requires N, 7·2%).

Nitration of Methyl and Ethyl Acenaphthyleno(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate*.—After nitric acid (0·28 c.c., *d* 1·5) had been added to a solution of the methyl ester (2 g.) in glacial acetic acid (25 c.c.) at 115°, a red solid (0·5 g.) rapidly appeared. When cold, this was recrystallised from acetic acid, and *methyl* 6(or 4)-*nitroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate* obtained in red needles, m. p. 243° (decomp.) (Found : N, 8·2. $C_{20}H_{12}O_4N_2$ requires N, 8·1%). After the mother-liquor from the reaction mixture had been left overnight at room temperature, a colourless solid (0·2 g.) had separated, and, on recrystallisation from benzene, *methyl* 3-*nitro*-2-*acetoxy*-2 : 3-*dihydroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate* was isolated in colourless prisms, m. p. 208° (decomp.) (Found : C, 65·0; H, 4·1; N, 6·6. $C_{22}H_{16}O_6N_2$ requires C, 65·3; H, 4·0; N, 6·9%). When the above reaction was carried out at 100°, the yield of the nitro-compound was greater, but no addition product crystallised from the mother-liquor.

Similar treatment of the ethyl ester (1 g.) in acetic acid (6 c.c.) at 80° led to the rapid separation of *ethyl* 6(or 4)-*nitroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate* (0·6 g.), golden yellow needles, m. p. 218—219°, from acetic acid (Found : N, 8·0. $C_{21}H_{14}O_4N_2$ requires N, 7·8%), while *ethyl* 3-*nitro*-2-*acetoxy*-2 : 3-*dihydroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*-1-*carboxylate* (0·3 g.), colourless prisms, m. p. 210—211° (decomp.), from alcohol (Found : C, 65·8; H, 4·4. $C_{23}H_{18}O_6N_2$ requires C, 66·0; H, 4·3%), crystallised from the mother-liquor when left for several hours at room temperature.

Nitration of 1-*Cinnamoylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*.—The cinnamoyl compound (2 g.) in acetic acid (50 c.c.) at 100°, on being similarly nitrated, yielded first 6(or 4)-*nitro*-1-*cinnamoylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole* (0·3 g.), orange-red needles, m. p. 261°, from nitrobenzene, identical (mixed m. p.) with the synthetical material described below (Found : N, 7·0. $C_{27}H_{16}O_3N_2$ requires N, 6·7%), and then a buff-coloured solid (0·7 g.), which, after several recrystallisations from benzene–light petroleum, gave an apparently pure *substance* in colourless prisms, m. p. 225° (decomp.) (Found : C, 70·4; H, 4·1; N, 5·9. $C_{29}H_{20}O_5N_2$ requires C, 70·7; H, 4·1; N, 5·7%).

Synthesis of 6(or 4)-*Nitro*-1-*cinnamoylacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*.—After an alcoholic solution of acenaphthenone (2 g.) and *m*-nitrophenylhydrazine (1·8 g.) had been boiled for 2 hours, the m-*nitrophenylhydrazone* (3·2 g.), red needles, m. p. 215° (decomp.) from *iso*amyl alcohol (Found : N, 13·7. $C_{18}H_{12}O_2N_3$ requires N, 13·9%), crystallised on cooling. A solution of the hydrazone (1·2 g.) in the minimum quantity of boiling alcohol was treated with concentrated hydrochloric acid (50 c.c.), the whole refluxed for 22 hours, and allowed to cool. The crude 6(or 4)-nitroacenaphthyleno(7′ : 8′ : 2 : 3)indole (0·9 g.), which separated, was dissolved in acetone (30 c.c.) and cinnamoylated by the method described for the indole itself. On recrystallisation of the product from nitrobenzene, 6(or 4)-nitro-1-cinnamoylacenaphthyleno(7′ : 8′ : 2 : 3)indole (0·8 g.) was obtained in orange-red needles, m. p. 261°, identical (mixed m. p.) with the substance mentioned above.

The 6(or 4)-nitro-derivatives of the acetyl, benzoyl, and carbomethoxy-compounds described above were hydrolysed by refluxing for an hour with aqueous-alcoholic potassium hydroxide, and the products, precipitated by water, cinnamoylated in the usual way. In each case the substance obtained was identical (mixed m. p.) with the above synthetical material.

Acenaphthenone o-*nitrophenylhydrazone*, red needles, m. p. 175°, from *iso*amyl alcohol (Found : N, 13·6%), was prepared like the *m*-isomer, but attempts to convert it into the corresponding indole were unsuccessful.

7-*Chloro*-4-*nitroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole*.—After a mixture of acenaphthenone (4 g.) and

2-chloro-5-nitrophenylhydrazine (4·5 g.) had been heated at 135—140° for ½ hour, the product was crystallised from *cyclo*hexanone, from which the *2-chloro-5-nitrophenylhydrazone* separated in yellow prisms (3·5 g.), m. p. 226° (decomp.) (Found : N, 12·2. $C_{18}H_{12}O_2N_3Cl$ requires N, 12·4%). When a solution of the hydrazone (2·7 g.) in a mixture of alcohol (200 c.c.) and concentrated hydrochloric acid (100 c.c.) was refluxed for 14 hours, *7-chloro-4-nitroacenaphthyleno*(7′ : 8′ : 2 : 3)*indole* (1·7 g.), orange-red prisms, m. p. 279—282°, from *cyclo*hexanone, gradually separated (Found : N, 9·3. $C_{18}H_9O_2N_3Cl$ requires N, 8·7%).

5-Bromoacenaphthyleno(7′ : 8′ : 2 : 3)*indole*.—Acenaphthenone (1 g.) and *p*-bromophenylhydrazine (1·1 g.) in boiling alcohol gave the p-*bromophenylhydrazone*, yellow prisms (1·6 g.), m. p. 185° (decomp.), from alcohol (Found : N, 8·5. $C_{18}H_{13}N_2Br$ requires N, 8·3%). It was refluxed with aqueous-alcoholic hydrochloric acid for 17 hours, and when the solid obtained by adding water was crystallised from benzene, *5-bromoacenaphthyleno*(7′ : 8′ : 2 : 3)*indole* (in 55% yield) separated in orange-yellow needles, m. p. 200° (Found : N, 4·3. $C_{18}H_{10}NBr$ requires N, 4·4%).

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247. *A Reaction of Certain Diazosulphonates derived from β-Naphthol-1-sulphonic Acid. Part XXVI. Phthalazine and isoIndolinone Compounds derived from Aniline.*

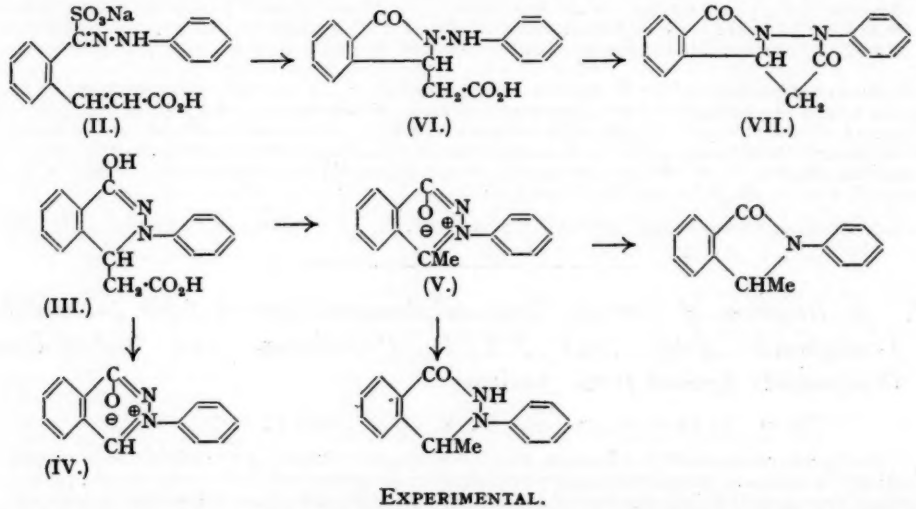
By A. T. PETERS, (the late) F. M. ROWE, and C. I. BRODRICK.

The parent compounds in this series are now prepared. Fission of the naphthalene ring in sodium 1-benzeneazo-β-naphthaquinone-1-sulphonate (I) occurs rapidly in presence of aqueous sodium hydroxide at 0°, but the sodium benzaldehydephenylhydrazone-ω-sulphonate-2-β-acrylic acid (II) formed is quickly converted into sodium hydrogen 3-phenyl-3 : 4-dihydrophthalazine-1-sulphonate-4-acetate. New technique is essential in order to ensure smooth hydrolysis to 1-*hydroxy*-3-*phenyl*-3 : 4-*dihydrophthalazine*-4-*acetic acid* (III). With restricted action of alkali hydroxide, (I) affords a little (II), hydrolysed to 2-anilino*iso*indolinone-3-acetic acid, the presence of which is confirmed by conversion into 2 : 5-*diketo*-3-*phenyl*iso*indolinopyrazolidocoline* (VII). [3-*Phenylphthalaz*-1-*one* (IV) and its 4-*methyl* derivative (V), unlike other analogues in this series, are water-soluble, and special methods are required for their preparation from compound (III).]

SODIUM hydrogen 3-phenyl-3 : 4-dihydrophthalazine-1-sulphonate-4-acetate was hydrolysed by adding a dilute ammonia solution to boiling dilute hydrochloric acid at such a rate as to ensure constant evolution of sulphur dioxide and minimum tar formation. The resulting 1-*hydroxy*-3-*phenyl*-3 : 4-*dihydrophthalazine*-4-*acetic acid* (III) is the most water-soluble of all such analogues prepared, and can be isolated only after concentration of the aqueous solution to a small volume. With boiling acetic anhydride, (III) gave the O-*acetate*, but on prolonged boiling (50 hours) with acetic anhydride and pyridine, the main product of *O*-acetate was accompanied by a little of the internal *anhydride* of (III). Sulphonation first occurs when compound (III) is heated with aqueous sulphuric acid, with probable subsequent formation of the sulphonic acid of (IV); the water-soluble 3-*phenylphthalaz*-1-*one* (IV) was, however, obtained in excellent yield by heating (III) with fuming hydrochloric acid (*d* 1·19) in a sealed tube at 180°, and it is not readily sulphonated. Further evidence for the constitution of (III) was afforded by (*a*) Clemmensen reduction to give *N*-phenylphthalimidine, which is also formed from 4′-amino-*N*-phenylphthalimidine by the diazo-reaction (cf. *J.*, 1926, 690), and (*b*) conversion of (III) by nitric acid (*d* 1·5) into the known 4′-nitro-3-phenyl-4-methylphthalaz-1-one. The phthalazone (IV) was not isomerized by heating it with aqueous hydrochloric acid (1 : 8) in a sealed tube at 180° for 50 hours, whereas when a nitro-group is present in the *p*-position in the 3-phenyl side-chain, transformation into the corresponding 4′-nitro-3-phenylphthalaz-4-one occurs (cf. *J.*, 1937, 90). Oxidation of (III) by sodium dichromate in aqueous sulphuric acid was accompanied by sulphonation, but the water-soluble 3-*phenyl*-4-*methylphthalaz*-1-*one* (V) was formed in 36% yield from (III) and chromic acid in acetic acid at 20°. Alkaline dithionite (hydrosulphite) reduction of (V) gave 1-*keto*-3-*phenyl*-4-*methyltetrahydrophthalazine*, whereas Clemmensen reduction yielded N-*phenyl*-3-*methylphthalimidine*.

When sodium 1-benzeneazo-β-naphthaquinone-1-sulphonate and aqueous sodium hydroxide were kept at 0° for 5 minutes, and the resulting sodium salt hydrolysed, the hydroxy-compound (III) was formed mainly, but about 5% of the isomeric 2-anilino*iso*indolinone-3-acetic acid (VI) was also present. Fractional crystallisation failed to effect a separation, but as (III) is acetylated by acetic anhydride to the soluble *O*-acetate, whereas (VI) loses water to yield the sparingly soluble 2 : 5-*diketo*-3-*phenyl*iso*indolinopyrazolidocoline* (VII), this reaction was used to show the presence of (VI). That compound (VII) possessed the constitution assigned to it was confirmed

by nitration to give the known 2′ : 4′-analogue of (VII), which is also derived from a similar reaction to that described above, using 2 : 4-dinitroaniline in place of aniline (cf. *J.*, 1947, 829).



(II.) (VI.) (VII.)

(III.) (V.)

(IV.)

Experimental.

1-*Hydroxy*-3-*phenyl*-3 : 4-*dihydrophthalazine*-4-*acetic Acid* (III).—Benzenediazonium chloride (from 100 g. of aniline), added to an ice-cold solution of 50% sodium β-naphthol-1-sulphonate (400 g.) in water (1200 c.c.), gave 2-naphthol-1-benzenediazosulphonate, which was salted out (sodium chloride), washed with brine, and converted by sodium carbonate (100 g.) in water (400 c.c.) at 0° into sodium 1-benzeneazo-β-naphthaquinone-1-sulphonate. After 15 minutes, sodium hydroxide (100 g.) in water (300 c.c.) at 5° was added to the mixture, and after 15 minutes, it was acidified by hydrochloric acid at below 10° to yield a black tar which did not solidify even after several days at 0°. The tar dissolved readily in aqueous ammonia (only a trace of benzeneazo-β-naphthol was formed), and careful addition to boiling dilute hydrochloric acid by means of a dropping-funnel during 6 hours, the mixture being kept acid to Congo-red, caused liberation of sulphur dioxide and gave an almost clear solution. Too rapid addition of the ammonia solution gave much black insoluble tar. Concentration of the resulting dilute hydrochloric acid solution gave successive crops of product, and final evaporation of the mother liquor to dryness, followed by extraction with ethyl acetate, afforded a further amount of 1-*hydroxy*-3-*phenyl*-3 : 4-*dihydrophthalazine*-4-*acetic acid* (III), which crystallised from acetic acid or ethyl acetate in colourless prismatic needles, m. p. 264° (decomp.) (191 g.; 63%) (Found : C, 68·2; H, 5·2; N, 10·25. $C_{16}H_{14}O_3N_2$ requires C, 68·1; H, 5·0; N, 9·9%).

The *ethyl* ester crystallised from alcohol in colourless rectangular prisms, m. p. 91—92° (Found : N, 8·8. $C_{18}H_{18}O_3N_2$ requires N, 9·0%). On being boiled with acetic anhydride for 3 hours, (III) gave the O-*acetate*, which crystallised from acetic acid in colourless plates, m. p. 183° (Found : N, 8·75. $C_{18}H_{16}O_4N_2$ requires N, 8·6%); when the boiling with acetic anhydride (and a little pyridine) was extended to 50 hours, the *O*-acetate was accompanied by *ca.* 4% of the internal *anhydride* of (III), m. p. > 320° (Found : N, 10·6. $C_{16}H_{12}O_2N_2$ requires N, 10·6%).

1-*Keto*-3-*phenyl*-2-*methyltetrahydrophthalazine*-4-*acetic acid* crystallised from ethyl acetate in colourless, prismatic needles, m. p. 184—186° (Found : C, 69·2; H, 5·35; N, 9·6. $C_{17}H_{16}O_3N_2$ requires C, 68·9; H, 5·4; N, 9·5%.)

3-*Phenylphthalaz*-1-*one* (IV).—1-Hydroxy-3-phenyl-3 : 4-dihydrophthalazine-4-acetic acid (7 g.) and fuming hydrochloric acid (*d* 1·19; 15 c.c.) in a sealed tube at 180° for 1 hour, followed by heating the resulting product with 15% aqueous sodium carbonate for 10 minutes, gave the *phthalazone* (IV), which crystallised from benzene–pyridine (1 : 1) in almost colourless needles, m. p. 208° (5·4 g.; 97%), which became green on keeping (Found : C, 76·0; H, 4·5; N, 12·5. $C_{14}H_{10}ON_2$ requires C, 75·8; H, 4·5; N, 12·6%). It formed a hydrochloride, which separated from water in long, colourless needles, m. p. 162—164°, and a *picrate*, which crystallised from alcohol in yellow prismatic needles, m. p. 226° (Found : N, 15·65. $C_{20}H_{13}O_8N_5$ requires N, 15·5%).

Methylation of (IV) with methyl sulphate at 80° gave a resin which combined with alcohol on boiling a solution for 5 minutes, when almost colourless prisms, m. p. 86—87°, separated (Found : C, 72·5; H, 6·3; N, 10·3. $C_{17}H_{16}O_2N_2$ requires C, 72·3; H, 6·4; N, 9·9%); this methylated *compound* lost alcohol when heated at 140° for 4 hours, and a *compound* was formed which crystallised from ethyl acetate in yellow needles, m. p. 234° (decomp.) [Found : N, 11·45. $(C_{15}H_{12}ON_2)_2$ requires N, 11·9%], probably resulting from the joining of two mols. (after loss of C_2H_6O) at the C_4 position. It differed from compound (V).

N-*Phenylphthalimidine*.—3-Phenylphthalaz-1-one (2 g.) was heated with hydrochloric acid (140 c.c.), water (80 c.c.), alcohol (40 c.c.), and zinc amalgam (200 g.) at 70—80° for 4 hours. The phthalimidine (1·6 g.; 85%) crystallised from alcohol in colourless plates, identical with an authentic specimen.

3-*Phenyl*-4-*methylphthalaz*-1-*one* (V).—Chromium trioxide (4 g.) in acetic acid (20 c.c.) and water (10 c.c.) was added slowly (during 2 hours) to a stirred suspension of finely-divided 1-hydroxy-3-phenyl-3 : 4-dihydrophthalazine-4-acetic acid (10 g.) in acetic acid (70 c.c.) at 20°. After standing overnight, the

precipitate was heated with 15% aqueous sodium carbonate and the residue of *phthalazone* (V) crystallised from benzene–pyridine in colourless rhombs, m. p. 235° (3·1 g.; 35·9%) (Found: C, 76·0; H, 5·0; N, 11·8. $C_{15}H_{12}ON_2$ requires C, 76·3; H, 5·1; N, 11·9%). 3-Phenyl-4-methylphthalaz-1-one gave a *picrate*, which crystallised from alcohol in yellow rhombs, m. p. 197° (Found: C, 54·6; H, 3·6; N, 14·8. $C_{21}H_{15}O_8N_5$ requires C, 54·2; H, 3·2; N, 15·05%).

1-*Keto*-3-*phenyl*-4-*methyltetrahydrophthalazine*.—Compound (V) (2 g.) was boiled with sodium dithionite (hydrosulphite) (4 g.) in dilute aqueous sodium hydroxide for 10 minutes, and the precipitate which separated on cooling was crystallised from aqueous alcohol. The *tetrahydrophthalazine* separated in colourless prisms, m. p. 171° (0·45 g.; 44·7%) (Found: C, 75·9; H, 5·8; N, 11·7. $C_{15}H_{14}ON_2$ requires C, 75·6; H, 5·9; N, 11·8%).

N-*Phenyl*-3-*methylphthalimidine*.—When 3-phenyl-4-methylphthalaz-1-one (3 g.), hydrochloric acid (70 c.c.), water (50 c.c.), and zinc amalgam (90 g.) were boiled for 4 hours, a resinous product was formed. On extracting with light petroleum, and cooling, long, colourless needles, m. p. 80°, of the *phthalimidine* separated (Found: C, 80·8; H, 5·6; N, 6·3. $C_{15}H_{13}ON$ requires C, 80·7; H, 5·8; N, 6·3%).

4′-*Nitro*-3-*phenyl*-4-*methylphthalaz*-1-*one*.—1-Hydroxy-3-phenyl-3 : 4-dihydrophthalazine-4-acetic acid (10 g.) was added during 30 minutes to nitric acid (*d* 1·5; 70 c.c.) at below 10°, and the mixture poured on ice, neutralised with aqueous ammonia, and kept overnight. The precipitate of 4′-nitro-3-phenyl-4-methylphthalaz-1-one nitrate (7·8 g.; 73·6%) was basified with warm aqueous ammonia, and the free base crystallised from methyl alcohol in yellow prismatic needles, m. p. 248° (decomp.), identical with an authentic specimen.

3-*Phenylphthalaz*-1-*one*-x-*sulphonic Acid*.—On being refluxed with aqueous sulphuric acid, b. p. 140°, or with equal volumes of sulphuric and acetic acid, for 40 minutes, and poured on ice, the hydroxy-compound (III) yielded a colourless precipitate of a *sulphonic acid* (Found: C, 52·8; H, 4·0; N, 8·7; S, 8·9. $C_{14}H_{10}O_4N_2S,H_2O$ requires C, 52·5; H, 3·75; N, 8·75; S, 10·0%). 3-Phenylphthalaz-1-one (IV) is not readily sulphonated under the above conditions, and thus sulphonation probably precedes phthalazone formation. The sulphonic acid, which does not melt, is probably a derivative of (IV).

3-*Phenyl*-4-*methylphthalaz*-1-*one*-x-*sulphonic Acid*.—1-Hydroxy-3-phenyl-3 : 4-dihydrophthalazine-4-acetic acid (10 g.) was added to concentrated sulphuric acid (100 c.c.) and the mixture poured on ice (250 g.); potassium dichromate (8 g.) was added during 30 minutes, with stirring, and, after 3 hours at room temperature, the resulting precipitate of *sulphonic acid* was collected (9·4 g.). It crystallised from a large volume of water in colourless, prismatic needles, which did not melt (Found: C, 56·9; H, 3·95; S, 9·6. $C_{15}H_{12}O_4N_2S$ requires C, 57·0; H, 3·8; S, 10·1%).

2 : 5-*Diketo*-3-*phenylisoindolinopyrazolidocoline* (VII).—A suspension of sodium 1-benzeneazo-β-naphthaquinone-1-sulphonate (from 50 g. of aniline) in aqueous sodium carbonate at 0° was added to sodium hydroxide (50 g.) in water (180 c.c.) at 0°, with vigorous stirring; the temperature was kept at 5°, and, after 5 minutes, the mixture was acidified quickly (hydrochloric acid and defrothing agent) without cooling. The resulting black mass of sodium salt was hydrolysed as described above, tar removed, and the acid solution evaporated to dryness. Extraction of the residue with ethyl acetate gave colourless prisms, m. p. 200° (softening at 180°) (22 g.). Fractionation from ethyl acetate afforded 1-hydroxy-3-phenyl-3 : 4-dihydrophthalazine-4-acetic acid (6·6 g.), m. p. and mixed m. p. 263—264°; the most soluble fraction (7·2 g.) was boiled with acetic anhydride (80 c.c.) for 5 minutes, and, on cooling, large colourless prisms (1·3 g.), m. p. 219°, separated. 2 : 5-*Diketo*-3-*phenylisoindolinopyrazolidocoline* (VII) crystallised from acetic anhydride or ethyl acetate in colourless prisms, m. p. 220—221° (Found: C, 72·5; H, 4·3; N, 10·1. $C_{15}H_{12}O_2N_2$ requires C, 72·7; H, 4·55; N, 10·6%).

Nitration of (VII).—Compound (VII) (0·5 g.) and nitric acid (*d* 1·5; 5 g.) were kept at room temperature for 10 minutes and then gradually warmed to 60° and poured on ice. The yellow solid crystallised from acetic acid in pale yellow prismatic needles, m. p. 238—239° (0·5 g.), not depressed on admixture with an authentic specimen of 2 : 5-diketo-3-(2′ : 4′-dinitrophenyl)*iso*indolinopyrazolidocoline (*J.*, 1947, 829).

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248. *The Preparation of the Halogenophenylacetic Acids.*

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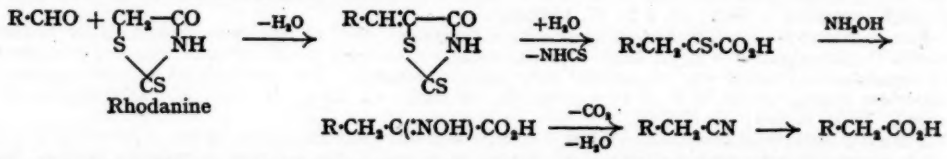
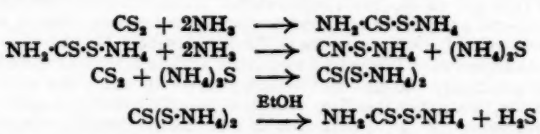
The chloro- and bromo-phenylacetic acids have been prepared by the rhodanine method and derivatives obtained. Other methods of preparation have also been examined. Attempts to separate the bromo-acids obtained by Bedson's method (*J.*, 1880, **37**, 94) by means of the acid chlorides, naphthalides, etc., were only partly successful. In the preparation of the β-naphthalides some ββ′-dinaphthylamine was obtained, but attempts to prepare this substance by Knoevenagel's second method (*J. pr. Chem.*, 1914, **89**, 23) yielded only *N*-aryl-β-naphthylamines.

SINCE no good method is available for the preparation of all the halogenophenylacetic acids it was decided to apply Grănacher's synthesis (*Helv. Chim. Acta*, 1922, **5**, 610; 1923, **6**, 458) the course of which is represented as follows:



By this means Julian and Sturgis (*J. Amer. Chem. Soc.*, 1935, **57**, 1126) and Plucker and Amstutz (*ibid.*, 1940, **62**, 1512) obtained excellent yields of other substituted acetic acids.

This method obviously depends on a satisfactory preparation of rhodanine and this at first presented much trouble. We followed the instructions of Julian and Sturgis (*loc. cit.*) by passing ammonia into carbon disulphide in a mixture of ethanol and ether, obtained rhodanine, and traced the frequent poor yield to the first stage of the reaction, *i.e.*, the preparation of ammonium dithiocarbamate. It has long been known that the above reactants may yield either ammonium trithiocarbonate or dithiocarbamate according to the conditions employed (Zeise, *Schweigger's J.*, 1824, **41**, 98; Debus, *Annalen*, 1850, **73**, 26; Mulder, *J. pr. Chem.*, 1868, **103**, 178; Freund and Bachrach, *Annalen*, 1895, **285**, 201). It was clear from the work of these authors that excess of ammonia favours formation of the trithiocarbonate, and excess of carbon disulphide that of the dithiocarbamate. The effect of temperature is not so clearly discernible. For example, Debus's statement (*loc. cit.*) that temperatures of 30—40° give mainly trithiocarbonate, and 10—15° dithiocarbamate, is seemingly not in agreement with Mulder's observation (*loc. cit.*) that at 30—40° he obtained only dithiocarbamate. The explanation is found in the ammonia concentration, which appears to be determinative, the more concentrated the ammonia solution the greater the tendency for trithiocarbonate formation. Ammonia, of course, is much more soluble in cold than in hot ethanol. Accordingly, when we passed ammonia through a " well-cooled alcoholic solution of carbon disulphide " (Julian and Sturgis, *loc. cit.*), *i.e.*, at about − 5°, the main product which separated was ammonium trithiocarbonate, but with the solution temperature maintained at 10—15° ammonium dithiocarbamate was obtained in fair yield. It is highly probable, however, that even under favourable conditions the dithiocarbamate is formed not only directly by interaction of carbon disulphide and ammonia, but also (with loss of yield) from the trithiocarbonate and ammonia according to the following reactions, each known to occur readily under appropriate conditions.



Although the conversion of benzaldehydes into phenylacetonitriles involves four stages, the overall yields are reasonably good, *viz.*, 57, 47, and 62% for the *o*-, *m*-, and *p*-chloro-, and 49, 44, and 38% for the corresponding bromophenylacetonitriles, respectively. Unfortunately, further losses, sometimes considerable, occur in the hydrolysis to the acids. The preparation of the halogenophenylacetic acids by the Grănacher method, therefore, leaves much to be desired. This is in agreement with Hahn and Schulz's findings on the preparation of methoxyphenylacetic acids (*Ber.*, 1939, **72**, 1302); they did not give any experimental details, but evidently encounted difficulties in the last two stages of the synthesis. Similarly, Barltrop (*J.*, 1946, 958) prepared 2 : 3-dimethoxyphenylacetonitrile in 24% yield from the benzaldehyde, the greatest losses occurring in the last two stages.

Efforts were made to separate the mixture of *o*- and *p*-bromophenylacetic acids obtained by Bedson's method (*loc. cit.*) by fractional distillation of the acid chlorides and ethyl esters. This being unsuccessful, chromatographic separation of the anilides, *p*-toluidides, and β-naphthalides was attempted in the hope that the process would be facilitated by the fluorescence of these compounds. Although they fluoresced in benzene they did not on the column, the only fluorescent bands being due to impurity. Chromatographic separation was therefore " blind " and only partly successful.

In the preparation of the naphthalides by heating the acids with β-naphthylamine, ββ-dinaphthylamine was also obtained. This substance is not obtained (unless in negligible quantities) when β-naphthylamine is heated alone, but with catalytic amounts of iodine excellent

yields result (Knoevenagel, *loc. cit.*). In our experiments the catalyst is presumably the halogenophenylacetic acid.

Knoevenagel (*loc. cit.*) also found that β-naphthylamine when heated with aromatic amines and a trace of iodine gives ββ′-dinaphthylamine. We have found this to be true for the halogenoanilines, but, with aniline and the toluidines, *N*-phenyl- or *N*-tolyl-β-naphthylamines are invariably obtained. We have not been able to explain this discrepancy between Knoevenagel's results and ours.

In our chromatographic work we noted that β-naphthylamine and its derivatives are more strongly adsorbed than the corresponding α-derivatives. This is in agreement with other observations. β-Benzoylnaphthalene is more strongly adsorbed than the α-compound (Campbell and Easton, unpublished results), and naphthalene dyes with amino- or hydroxy-groups in the β-position are more strongly adsorbed from aqueous solutions on alumina than the α-isomers (Ruggli and Jensen, *Helv. Chim. Acta*, 1935, **18**, 624; 1936, **19**, 64). How far this rule holds remains to be determined, as adsorption is affected by the nature of the solvent, position of other substituents, etc. (cf. Ruggli and Jensen, *loc. cit.*, 1936).

Experimental.

Unless otherwise stated, the preparation, purification, and properties of substances are those given in the literature, and the chromatographic separations were carried out on alumina (Brockmann) with benzene as solvent and developer. The purity of all products was checked by the sharpness of their m. p.s on a Kofler or a Fuchs micro-m. p. apparatus (*Mikrochem.*, 1934, **15**, 242; *Mikrochim. Acta*, 1937, **2**, 317). The analyses were done by Drs. Weiler and Strauss, Oxford.

Preparation of Rhodanine.—Ammonia from a cylinder was passed fairly rapidly into a stirred mixture of carbon disulphide (250 g.), alcohol (200 c.c.), and ether (200 c.c.), the temperature being kept at 5—15° (but not lower) by ice–water. After 4 hours 170 g. of ammonium dithiocarbamate had separated and was added to a solution of sodium chloroacetate, prepared according to Julian and Sturgis (*loc. cit.*), equivalent to 146 g. of chloroacetic acid. A further 65 g. of ammonium dithiocarbamate were obtained by passing ammonia into the filtrate for 2 hours. This was added to another portion of chloroacetate solution equivalent to 56 g. of the acid. The solutions were combined and after an hour were run into 302 c.c. of 10N-hydrochloric acid heated to 80—90° and kept at this temperature during the addition. The solution was kept overnight, and the rhodanine separated, washed with water, and dried in air; yield, 125 g. (44% calc. on ammonium dithiocarbamate); m. p. 165—170°.

In many experiments when the temperature was kept below 0° ammonium trithiocarbonate was the main product.

Ammonium trithiocarbonate and dithiocarbamate are distinguished in several ways. The former with a solution of sodium chloroacetate followed by acidification in the cold gives carboxymethyl trithiocarbonate, $CS(S{\cdot}CH_2{\cdot}CO_2H)_2$, bright yellow crystals, m. p. 172°, and the latter, thiocarbamylthio-glycollic acid, $NH_2{\cdot}CS{\cdot}S{\cdot}CH_2{\cdot}CO_2H$, pale yellow crystals, m. p. 137°. The trithio-compound rapidly turns orange then red and finally decomposes on exposure to air, though these changes occur less readily if the substance is purified by washing with alcohol; but the dithio-compound is stable under these conditions. Decisive colour tests are also available. A few mg. of the substance in two drops of sodium hydroxide are treated with two drops of a freshly prepared sodium nitroprusside solution. The trithiocarbonate gives a red colour and the dithiocarbamate a blue deepening to violet: dithiocarbamate with some trithiocarbonate gives a purple shade. Finally, the trithiocarbonate with solutions of stannous chloride and lead acetate gives dark red and bright red precipitates respectively; dithiocarbamate gives buff and yellow precipitates.

Preparation of Benzylidenerhodanines.—Molecular quantities of aldehyde and rhodanine were dissolved in glacial acetic acid (5 c.c. per g. of aldehyde) and refluxed in an oil-bath with fused sodium acetate (twice the weight of rhodanine) for half an hour. The product was washed with a little glacial acetic acid, well with water, and dried at 70°; yield, 80—97%. These rhodanines were recrystallised from glacial acetic acid. *o*-Chlorobenzylidenerhodanine, pale yellow needles, m. p. 192°; yield, 97% (Found: Cl, 12·8. Calc. for $C_{10}H_6ONClS_2$: Cl, 13·9%). Andreasch (*Monatsh.*, 1928, **49**, 132) obtained the substance as brown-red needles, m. p. 169°, which were obviously impure. *m-Chlorobenzylidene-rhodanine*, pale yellow elongated prisms, m. p. 233°; yield, 93% (Found: Cl, 14·1%). *p-Chloro-benzylidenerhodanine*, yellow needles, m. p. 231—232°; yield, 93% (Found: Cl, 14·0%). *o-Bromo-benzylidenerhodanine*, orange needles, m. p. 183·5°; yield, 80% (Found: Br, 22·6. $C_{10}H_6ONBrS_2$ requires Br, 26·7%); repeated analyses were also unsatisfactory, but the impure substance gave pure products in the later stages of the synthesis. *m-Bromobenzylidenerhodanine*, yellow needles, m. p. 238°; yield, 90% (Found: Br, 26·1%). *p-Bromobenzylidenerhodanine*, yellow elongated prisms, m. p. 237—238°; yield, 84% (Found: Br, 27·9%).

Preparation of β-Phenylthiopyruvic Acids.—The procedure of Julian and Sturgis (*loc. cit.*) proved unsatisfactory and the following modification was used. The benzylidenerhodanine was cleaved with 8% sodium hydroxide (8 c.c. per g. of benzylidene compound) by heating in a water-bath at 50—55°. The mixture was well stirred until a clear or nearly clear solution was obtained. The solution was filtered if necessary, cooled in an ice–salt mixture, and acidified *rapidly* with 3N-hydrochloric acid with stirring. The stirring and cooling were continued for 10 minutes, and the acid thus obtained washed with water and dried in a vacuum over calcium chloride. Crystallisation of the acids was difficult (cf. Grănacher, *loc. cit.*), but a good yield of pure acid was obtained by dissolving the crude substance in the minimum quantity of cold alcohol, filtering, and adding 1—2 times the volume of cold water with stirring and shaking. After 2 hours the acid was dried in a vacuum over calcium chloride. Further purification was effected by crystallisation from methanol, light petroleum, etc., but the crude acids were used for

the next stage in the synthesis. *β-o*-Chlorophenyl acid, prisms (methanol), m. p. 134—135°; yield, 72% (Found: S, 15·3. Calc. for $C_9H_7O_3ClS$: S, 14·9%); further crystallisation from light petroleum (b. p. 100—120°) gave needles, m. p. 142—143°. Andreasch (*loc. cit.*) gives m. p. 119—120°. *m-Chlorophenyl acid*, straw-coloured needles (light petroleum, b. p. 100—120°), m. p. 134°; yield, 84% (Found: S, 14·7%). *p*-Chlorophenyl acid, m. p. 164—167°; yield, 84% (Found: S, 15·1%); recrystallisation from ethanol gave yellow prisms, m. p. 169—171°, which resolidified, and melted finally at 202—204°. Gendelman (*Monatsh.*, 1922, **43**, 537) obtained it as pale yellow needles (alcohol), m. p. 157°. An oil was also obtained which on trituration with aqueous alcohol gave a product only slightly soluble in *iso*propyl alcohol. It separated from a large volume of boiling ethanol in yellow prisms, m. p. 221—222°, and is probably *bis*-(m-*chlorocinnamic acid*) *α-disulphide*, $[C_6H_4Cl{\cdot}CH{:}C(CO_2H){\cdot}S{-}]_2$ (Found: C, 50·9; H, 3·0; Cl, 17·3; S, 15·1. $C_{18}H_{12}O_4Cl_2S_2$ requires C, 50·6; H, 2·8; Cl, 16·6; S, 15·1%). o-*Bromophenyl acid*, m. p. 134—139°, yield, 70%; separated from light petroleum (b. p. 100—120°) in lemon-yellow elongated prisms, m. p. 142—143° (Found: S, 11·3. $C_9H_7O_3BrS$ requires S, 12·4%). m-*Bromophenyl acid*, pale yellow needles (light petroleum, b. p. 100—120°), m. p. 133—134°, yield, 81% (Found: S, 12·7%). p-*Bromophenyl acid*, m. p. 165—180°, yield, 75% (Found: S, 11·45%); efforts to purify this substance further were unsuccessful.

Preparation of α-Oximino-β-phenylpropionic Acids.—The most satisfactory results were obtained by refluxing the thioketo-acids with an alcoholic solution containing 3 mols. of hydroxylamine. The calculated quantity of hydroxylamine hydrochloride was dissolved in the minimum amount of warm water and added to 2·5N-alcoholic sodium hydroxide, sufficient to liberate the base. Alcohol was then added to bring the volume of solution to 10 c.c. per g. of thioketo-acid. The sodium chloride was removed, the thioketo-acid added, and the solution refluxed until no more hydrogen sulphide was evolved (about ½ hour). The alcohol was removed by vacuum distillation from a water-bath, the residue taken up in dilute sodium hydroxide, the solution filtered, and the filtrate acidified with concentrated hydrochloric acid. The oximino-acid was well washed with water, and dried in a vacuum over potassium hydroxide. The crude acid was used for the next stage of the synthesis, but a sample was crystallised for analysis. No attempt was made to isolate the *cis*- and *trans*-oximino-acids.

α-Oximino-β-halogenophenylpropionic acids: o-*Chlorophenyl-*, yield, 83%, m. p. 146—148°, raised by crystallisation from aqueous ethanol to 156° (Found: N, 6·78. $C_9H_8O_3NCl$ requires N, 6·56%). m-*Chlorophenyl-*, m. p. 145—148°, yield, 100%; colourless needles (benzene), m. p. 149° (decomp.) (Found: N, 6·74%). p-*Chlorophenyl-*, most samples had m. p. 170° (decomp.), but one had m. p. 182°; yield, 100% (Found: N, 6·17%). o-*Bromophenyl-*, m. p. 150°, yield, 100%; colourless needles (benzene), m. p. 150° (decomp.) (Found: N, 5·34. $C_9H_8O_3NBr$ requires N, 5·43%). m-*Bromophenyl-*, m. p. 151°, yield, 93%; colourless crystals (benzene), m. p. 151° (Found: N, 5·47%). p-*Bromophenyl-*, m. p. 168—169°, yield, 85%; elongated prisms (toluene), m. p. 173° (Found: N, 5·28%).

Halogenophenylacetonitriles.—The crude oximino-acid was added to acetic anhydride (4 c.c. per g. of acid), and the mixture warmed gently under reflux. The flame was removed as soon as a vigorous reaction occurred, and after this had ceased the mixture was refluxed for 10 minutes. The acetic anhydride was removed by distillation under reduced pressure from a water-bath, and the residue extracted with ether, which was then washed with sodium carbonate solution and finally with water. The solution was dried ($CaCl_2$), the ether removed by distillation, and the residue distilled in a vacuum. *o*-Chlorophenylacetonitrile, b. p. 123—125°/11 mm., yield, 64% (Found: N, 9·75. Calc. for C_8H_6NCl: N, 9·25%). *m*-Chlorophenyl-, b. p. 134—136°/10 mm., yield, 55% (Found: N, 9·80%). *p*-Chlorophenyl-, b. p. 137—139°/12 mm., colourless needles (light petroleum, b. p. 40—60°), m. p. 31—32° (lit., 29—30°), yield, 80% (Found: N, 9·5%). *o*-Bromophenyl-, b. p. 140—141°/13 mm., yield, 88% (Found: N, 7·88. Calc. for C_8H_6NBr: N, 7·14%). *m*-Bromophenyl-, b. p. 145—147°/10 mm., yield, 70%; needles (light petroleum, b. p. 40—60°), m. p. 27—28° (Found: N, 7·52%). *p*-Bromophenyl-, b. p. 152—156°/10—12 mm., yield, 72%; lamellæ (light petroleum, b. p. 40—60°), m. p. 48° (lit., 47°) (Found: N, 7·07%).

Halogenophenylacetic Acids.—The nitriles were hydrolysed by boiling with 60% sulphuric acid or preferably 20% alcoholic potassium hydroxide. *o*-Chlorophenylacetic acid, m. p. 93—95° (lit., 96°) (Found: Cl, 19·6. Calc. for $C_8H_7O_2Cl$: Cl, 20·84%) [p-*nitrobenzyl* ester, prisms (ethanol), m. p. 70—71° (Found: N, 4·51. $C_{15}H_{11}O_4NCl$ requires N, 4·57%)]. *m*-Chlorophenylacetic acid, plates (aqueous ethanol), m. p. 77° (lit., 76°) (Found: Cl, 21·1%) [p-*nitrobenzyl* ester, colourless crystals (ethanol), m. p. 74—75° (Found: N, 4·87%); p-*toluidide*, crystals (dilute acetic acid), m. p. 138° (Found: N, 5·62. $C_{15}H_{14}ONCl$ requires N, 5·4%)]. *p*-Chlorophenylacetic acid, needles (light petroleum, b. p. 80—100°), m. p. 104—106° (lit., 105°) (Found: Cl, 21·7%) [p-*nitrobenzyl* ester, elongated prisms (ethanol), m. p. 117° (Found: N, 4·77%)]. *o*-Bromophenylacetic acid, m. p. 104—105° (lit., 105—106°; 109°), needles (light petroleum, b. p. 80—100°), identical with a specimen prepared from *o*-bromobenzoyl chloride by the Arndt–Eistert method (Fieser and Kilmer, *J. Amer. Chem. Soc.*, 1940, **62**, 1356) [p-*nitrobenzyl* ester, plates (methanol), m. p. 74—75° (Found: N, 4·4. $C_{15}H_{12}O_4NBr$ requires N, 4·0%); p-*toluidide*, elongated prisms (glacial acetic acid), m. p. 183—184° (Found: N, 4·8. $C_{15}H_{14}ONBr$ requires N, 4·61%); ethyl ester, elongated prisms (light petroleum, b. p. 40—60°), m. p. 38—39° (lit., 35—36°) (Found: Br, 33·1. Calc. for $C_{10}H_{11}O_2Br$: Br, 32·9%); *anilide*, elongated prisms (ethanol), m. p. 153—154° (Found: Br, 27·6. $C_{14}H_{12}ONBr$ requires Br, 27·6%)]. *m*-Bromophenylacetic acid, needles (water), m. p. 102—103° (lit., 100°) (Found: Br, 37·0. Calc. for $C_8H_7O_2Br$: Br, 37·2%) [p-*nitrobenzyl* ester, crystals (methanol), m. p. 75—76° (Found: N, 4·3%); p-*toluidide*, crystals (dilute acetic acid), m. p. 135° (Found: N, 4·7%)]. *p*-Bromophenylacetic acid, m. p. 113—115° (lit., 114°), identical with a specimen prepared by the method of Czaplicki, Kostanecki, and Lampe (*Ber.*, 1909, **42**, 828) (see below) [p-*nitrobenzyl* ester, elongated prisms (ethanol), m. p. 128—129° (Found: N, 4·25%); p-*toluidide*, prisms (benzene), m. p. 203° (Found: N, 4·66%); *anilide*, m. p. 174—176° (Found: N, 4·75%. $C_{14}H_{12}ONBr$ requires N, 4·82%)].

p-Bromobenzaldehyde (20 g.), a freshly prepared solution of sodium hydrogen sulphite (80 c.c.), and ethanol (5 c.c.) yielded, on shaking, the crystalline bisulphite derivative, which was washed with several portions of cold water, then made into a paste with water, and stirred for 2 hours with potassium cyanide

(10 g.) in water (20 c.c.). Extraction with ether followed by evaporation yielded p-*bromomandelonitrile*, m. p. 60—70° (12·5 g.). A sample crystallised twice from benzene–light petroleum (b. p. 60—80°) had m. p. 78—79° (Found: N, 6·4. C_8H_6ONBr requires N, 6·6%). The nitrile (11 g.) was refluxed for 1 hour with 46 c.c. of hydriodic acid (*d* 1·94), the mixture poured into sodium hydrogen sulphite solution, and the resulting oil extracted with ether, which afforded, with sodium hydroxide followed by acidification, *p*-bromophenylacetic acid, m. p. 110—111° (yield, 1·5 g.); recrystallisation from light petroleum (b. p. 80—100°) and then aqueous ethanol gave m. p. 111·5—114° (Found: Br, 37·2. Calc. for $C_8H_7O_2Br$: Br, 36·8%). The ethereal layer gave a substance of unknown constitution which was crystallised from benzene; m. p. 126—127° (Found: C, 45·8; H, 3·33; N, 4·24; Br, 36·0%).

Phenylacetonitriles from Mandelonitrile Benzoates.—The method of Kindler and Peschke (*Arch. Pharm.*, 1933, **271**, 435) and Kindler and Gehlhaar (*ibid.*, 1936, **274**, 377) was investigated, platinum-black prepared by the method of Willstätter and Waldschmidt-Leitz (*Ber.*, 1921, **54**, 123) and tetralin purified by repeated shaking with concentrated sulphuric acid being used. *o*-Chlorobenzaldehyde (20 g.) was converted into *o*-chloromandelonitrile, which was then added to benzoyl chloride (27 g.) in pyridine (100 c.c.), and the solution kept overnight. Addition of water gave an oil which was extracted with ether and the ethereal layer washed with dilute hydrochloric acid, sodium carbonate, and finally water, and then dried ($CaCl_2$). On evaporation *o-chloromandelonitrile benzoate* was obtained as an oil (Found: N, 5·18. $C_{15}H_{10}O_2NCl$ requires N, 5·15%) which solidified after several weeks. Refluxing with platinum-black and tetralin resulted in removal of halogen as hydrogen chloride and no *o*-chlorophenylacetonitrile could be isolated. The method, however, was successfully applied to *p*-methoxyphenylacetonitrile benzoate, best prepared as follows. Anisaldehyde (20 g.) was added to sodium cyanide (15 g.) in water (20 c.c.), and benzoyl chloride (22 g.) added with vigorous shaking or stirring during 15 minutes, the temperature being kept at 35—40°. In an hour the oil began to solidify, a few drops of benzoyl chloride and a little sodium hydroxide were added, and stirring was continued 10 minutes longer. The pasty mass was triturated with dilute sodium hydroxide, and then washed successively with dilute sodium hydroxide, water, saturated sodium bisulphite, and water. Trituration with a little ethanol and drying in a vacuum gave a solid, m. p. 64—65° (yields in two runs 74 and 92%), from which *p*-methoxyphenylacetonitrile, b. p. 153—154/14 mm. (39% yield), was obtained.

Other methods were tried without much success. Catalytic reduction (platinised charcoal activated by palladium) of mandelic acids and nitriles (Zelinsky, Packendorff, and Leder-Packendorff, *Ber.*, 1934, **67**, 300) proved capricious; the azlactone method and the Willgerodt reaction gave poor yields; and the Arndt–Eistert synthesis, though giving reasonably good yields, was limited by slow formation of the intermediate diazoketone and the difficulty in working with diazomethane.

Separation of Bromophenylacetic Acids.—The mixture of bromophenylacetic acids (17 g.) obtained by Bedson's method (*loc. cit.*) was esterified with ethanol and concentrated sulphuric acid, and the product fractionally distilled in a vacuum twice, the final fraction, b. p. 164—169°/55 mm. (12·5 g.), being collected. This proved to be a mixture, since hydrolysis gave an oil from which with difficulty a little *o*-bromophenylacetic acid, m. p. 109°, was obtained. When the mixture of esters was kept at room temperature it partly solidified, the crystals (3·5 g.), m. p. 27°, being ethyl *o*-bromphenylacetate (no depression with an authentic sample; and hydrolysis to *o*-bromophenylacetic acid, m. p. 108°).

The mixture of acids and thionyl chloride yielded a mixture of acid chlorides, b. p. 145—155°/47 mm., 130—146°/42 mm., which gave *o*-and *p*-bromophenylacet-*p*-toluidides (separated as below).

The mixture of acids was converted into the toluidides by heating with *p*-toluidine for ½ hour. From the product, samples of *o*- and *p*-bromophenylaceto-*p*-toluidides were isolated by making use of the smaller solubility in boiling ethanol or benzene and the greater adsorbability on alumina of the latter compound. Quantitative separation, however, is very tedious.

The β-naphthalides were prepared from the mixture either of acid chlorides or of acids. The product of the first method was separated chromatographically with difficulty into the two isomers identical with samples prepared from the acid chlorides and β-naphthylamine. *o-Bromophenylaceto-β-naphthalide*, needles (benzene or methanol), m. p. 188—189° (Found: Br, 23·8. $C_{18}H_{14}ONBr$ requires Br, 23·5%), and p-*bromophenylaceto-β-naphthalide*, crystals (aqueous ethanol), m. p. 203—204° (Found: N, 3·9. $C_{18}H_{14}ONBr$ requires N, 4·11%), fluoresce in benzene but not on the chromatographic column, and the same holds for *phenylaceto-β-naphthalide*, colourless plates (ethanol), m. p. 162—163° (Found: N, 5·4. $C_{18}H_{15}ON$ requires N, 5·2%). In the second process the mixed acids (3 g.) were heated with β-naphthylamine (6 g.) in an oil-bath at 220° for ½ hour, the mixture poured into dilute hydrochloric acid, and the precipitate repeatedly extracted with warm dilute hydrochloric acid. 0·9 G. of the residue was dissolved in benzene (100 c.c.) and chromatographed on alumina (8″ × 1·3″) and developed with benzene–light petroleum (1 : 1 by vol.). From the filtrate 0·45 g. of colourless plates (light petroleum, b. p. 100—120°), m. p. 173—174°, was obtained and shown to be ββ′-dinaphthylamine by mixed m. p. with an authentic sample. It had the same brilliant livid blue fluorescence in the solid state and bright violet in benzene as a specimen prepared by heating β-naphthylamine with a trace of iodine (Knoevenagel, *loc. cit.*). In attempts to prepare this compound by heating β-naphthylamine and aniline with a trace of iodine (Knoevenagel, *loc. cit.*) the product, in spite of many variations of the conditions, was invariably *N*-phenyl-β-naphthylamine, m. p. 106—107° (lit., 108°); yield, 95%. *p*-Toluidine similarly gave *N*-*p*-tolyl-β-naphthylamine, m. p. 102—103° (lit., 102—103°), after chromatographic purification (yield, 95%), but *o*- or *m*-chloroaniline gave only ββ′-dinaphthylamine.

Chromatographic Separations.—α- and β-Naphthylamines (1 g. of each) were dissolved in benzene and passed through a column of alumina (1·7″ × 8″) and developed with benzene–light petroleum (4 : 1 by vol.). From the first 500 c.c. of filtrate α-naphthylamine (acetyl derivative, m. p. 155°) was obtained, and from the next 300 c.c. β-naphthylamine, m. p. 112° (mixed m. p.). Similarly, phenylaceto-β-naphthalide was more strongly adsorbed than the α-isomer (needles from benzene, m. p. 166—167°) (Found: 5·3. Calc. for $C_{18}H_{15}ON$: N, 5·2%).

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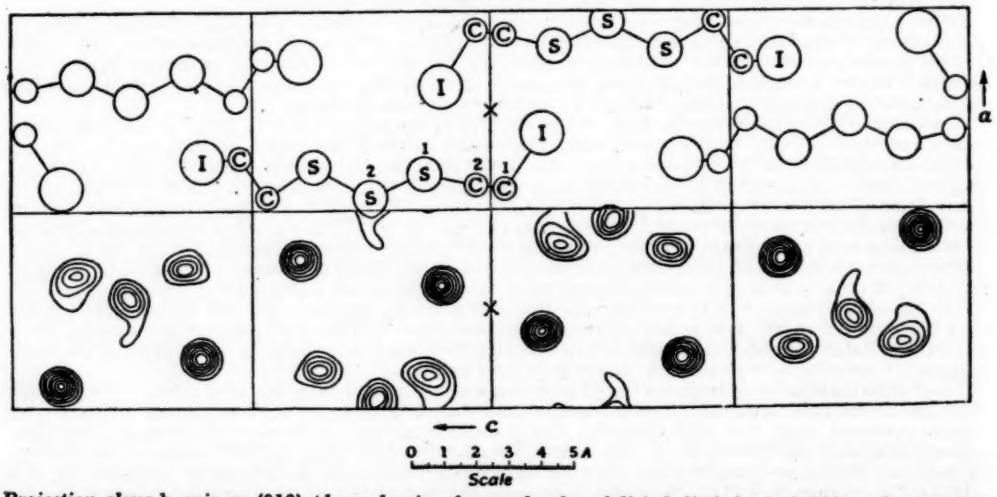
249. *The Structure of Certain Polysulphides and Sulphonyl Sulphides. Part II. The Crystal Structure of 2 : 2′-Di-iododiethyl Trisulphide.*

By I. M. Dawson and J. Monteath Robertson.

The crystal and molecular structure of 2 : 2′-di-iododiethyl trisulphide have been determined by quantitative *X*-ray analysis. There are four molecules in the unit cell and each molecule possesses an exact two-fold axis of symmetry (tetragonal space-group $P4_12_1$). Fourier projections used to refine the trial structure give good definition of the iodine and sulphur positions, but not of the carbon positions. The latter positions may, however, be inferred with reasonable certainty from the known bond lengths and valency angles involved. The molecule is in the form of an extended zigzag, but the planes of the carbon–carbon–iodine zigzags are almost at right angles to the plane of the central sulphur zigzag. The sulphur–sulphur distance is 2·04 ± 0·04 A. and the S–S–S angle is about 113°. The intermolecular distances found are in good agreement with those previously reported for sulphur and iodine, the van der Waals radius for iodine being 2·22 A. and for sulphur 1·58 A. The latter value is considerably less than the sulphur ionic radius (1·85 A.).

It is clear from the preliminary survey given in Part I (Dawson, Mathieson, and Robertson, *J.*, 1948, 322) that the crystal structure of 2 : 2′-di-iododiethyl trisulphide is suitable for detailed *X*-ray analysis, and a full determination is given in the present paper. The tetragonal space group D_4^4–$P4_1/2_1$ with four molecules in the unit cell ($a = 6{\cdot}01$, $c = 29{\cdot}4$ A.) demands that each molecule should possess a two-fold axis of symmetry, perpendicular to the (110) crystal plane.

Fig. 1.



Projection along b *axis on* (010) *plane showing four molecules of di-iododiethyl trisulphide. Contour lines for iodine atom are drawn at density intervals of* 10 *electrons per* A.², *and for sulphur at intervals of* 4 *electrons per* A.². *Origin of co-ordinates marked by* X.

This fact limits the possible molecular configurations to some extent, and effectively rules out any structure showing co-ordination of the type —S(↑S)—.

The very long *c* axis and relatively small cross-section, as well as pronounced cleavage parallel to (100), suggest rod-shaped molecules arranged approximately parallel to the *c* axis. This is in favour of an extended zigzag chain structure, although alternatives (VII) and (VIII) (Part I) cannot be finally eliminated from space-group and dimensional evidence alone.

In this structure the contribution of the iodine atoms to the *X*-ray intensities far outweighs that of all the other atoms combined. The distribution of scattering power in the molecule $C_4H_8S_3I_2$, obtained by summing the atomic numbers, is 57% for iodine, 26% for sulphur, and only 13% for carbon. The situation is clearly not such a favourable one for the application of the heavy-atom technique as in the case of platinum phthalocyanine (Robertson and Woodward, *J.*, 1940, 36) or cholesteryl iodide (Carlisle and Crowfoot, *Proc. Roy. Soc.*, 1945, *A*, 184, 64). In the present example there are too many heavy atoms and too few light ones. This is also

reflected in the absorption coefficient which, for copper radiation, is about five times higher in the present crystal than in the crystal of platinum phthalocyanine. Because of these factors, the final Fourier projection obtained in this analysis (Fig. 1) fails to give resolution of the carbon atoms, although their positions may be inferred with reasonable certainty from the positions of the other atoms.

The heavy atoms do not occupy any special positions in the structure, and although a Patterson analysis might be feasible, the dimensional evidence mentioned above made it more profitable to proceed by trial and error methods. Certain outstanding features in the (*h*0*l*) series of reflections help to simplify the problem. Large structure factors were observed for the (008), (106), (1014), (304), and (30, 24) planes, and when traces of these planes are drawn on the *ac* projection it becomes possible to define the probable positions of the iodine atoms. On this basis various trial models were set up and atomic positions were found which gave a satisfactory account of all the stronger reflections.

From this point the work proceeded by the application of double Fourier series methods, utilising the (*h*0*l*) structure factors. The projection first obtained gave clear resolution of the iodine positions, but the sulphur co-ordinates were rather doubtful. However, the accurate iodine co-ordinates now enabled a more complete Fourier series to be set up, and after several refinements of this kind the diagram shown in Fig. 1 was obtained. This projection, drawn on the (010) plane, covers one complete unit cell, and includes four molecules. The line diagram (upper portion of Fig. 1) indicates the molecular arrangement, and here probable positions for the carbon atoms are shown, although no direct evidence for these positions can be obtained from the electron density contours.

Co-ordinates and Dimensions.

The projection of the structure shown in Fig. 1 allows the co-ordinates of the iodine and sulphur atoms to be determined, the tetragonal symmetry making possible the direct measurement of all three co-ordinates for each atom. Table I lists co-ordinates for the atoms in the asymmetric crystal unit, which is one-half of the chemical molecule, or one-eighth of the total content of the unit cell. The co-ordinates of the remaining atoms follow from the symmetry operations of the space-group, D_4^4 ($P4_12_1$).

In this connection we have found it convenient to employ a different origin of co-ordinates from that adopted in the " International Tables for the Determination of Crystal Structure " (Bell and Son, London, 1935, p. 196). There the origin is taken at the intersection of one of the two-fold rotation axes, perpendicular to (110), with a two-fold screw axis parallel to *c*. For projections along the *c* axis this will give an origin at an apparent centre of symmetry. In the present example, however, the only useful projection of the structure is obtained along the *b* axis, and it is therefore convenient to choose an origin at an apparent centre of symmetry in this projection. Such a point is obtained on a two-fold screw axis parallel to *b* and is conveniently chosen at $(-a/4, -b/4)$ with respect to the tetragonal screw axis. This new origin is employed in the present paper, and it is situated at $(a/4, 3b/4, c/8)$ with respect to the origin adopted in the International Tables. The co-ordinates of the eight equivalent points then become

(1) $x, \quad y, \quad z$	(4) $y, \quad \frac{1}{2}-x, \frac{1}{2}+z$	(7) $\frac{1}{2}+y, \frac{1}{2}+x, \frac{1}{2}-z$
(2) $\frac{1}{2}-y, x, \quad \frac{1}{2}+z$	(5) $-y, \quad -x, \quad \frac{1}{2}-z$	(8) $-x, \quad \frac{1}{2}+y, -z$
(3) $\frac{1}{2}-x, \frac{1}{2}-y, \frac{1}{2}+z$	(6) $\frac{1}{2}+x, -y, \quad \frac{1}{2}-z$	

with four special positions at

(1) $x, \quad x, \frac{1}{8}$	(3) $\frac{1}{2}-x, \frac{1}{2}+x, \frac{1}{8}$
(2) $\frac{1}{2}+x, x, \frac{1}{8}$	(4) $-x, \quad \frac{1}{2}-x, \frac{1}{8}$

This treatment yields a simplified expression for the geometrical structure factor for the (*h*0*l*) reflections. In the usual notation this becomes

$$A = 4\left[\cos 2\pi\left(hx - \frac{h+l}{4}\right).\cos 2\pi\left(lz + \frac{h+l}{4}\right) + \cos 2\pi\left(hy - \frac{h-l}{4}\right).\cos 2\pi\left(lz + \frac{h+2l}{4}\right)\right]$$

$$B = 0$$

The expression may be simplified further for special values of the indices *h* and *l*.

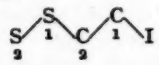
TABLE I.

Co-ordinates with respect to tetragonal crystal axes.

[Origin on 2_1 axis parallel to b, at $(-a/4, -b/4)$ from 4_1 axis.]

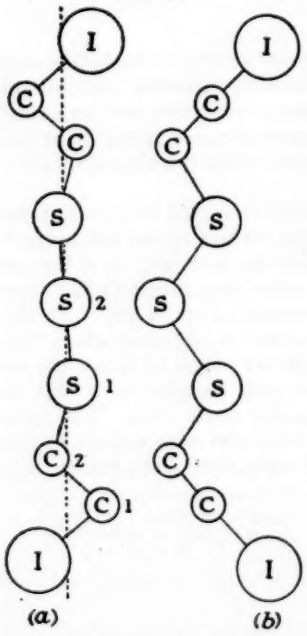
Atom (cf. Fig. 1).	x, A.	$\frac{2\pi x}{a}$.	y, A.	$\frac{2\pi y}{b}$.	z, A.	$\frac{2\pi z}{c}$.
I	−0·694	− 41·7°	1·500	90°	−1·531	−18·7°
C_1	−2·4	−144	1·4	84	−0·4	− 4·9
C_2	−2·3	−138	2·68	161	0·45	5·5
S_1	−1·96	−117·5	1·81	108·5	1·98	24·2
S_2	−2·68	−160·7	2·68	160·7	3·675	45

No clearly-defined carbon peaks occur in the Fourier projection and the carbon co-ordinates listed above are probable values only, based on the requirements of accepted single-bond lengths and angles for the atoms in question. In particular, the measured distance between the sulphur atom S_1 and the iodine atom in the chain is only 3·74 A. Assuming normal single-bond values, a *trans*-planar configuration for the group



would require a sulphur–iodine distance of 4·65 A. The difference is too great to be attributed to any minor bond-length or bond-angle variations, and the *trans*-planar configuration is therefore unlikely. The iodine atoms are also found to lie somewhat out of the plane of the S–S–S zigzag.

FIG. 2.



(a) *Projection of the molecule along the axis of symmetry.*
(b) *A projection at right angles to* (a).

The positions which we have assigned to the carbon atoms give reasonable values for all the bond lengths and bond angles in which they are involved, and also provide a maximum clearance between the carbon atom C_2 and the iodine atom on the neighbouring molecule at equivalent position (8), $-x$, $\frac{1}{2}+y$, $-z$. The van der Waals approach distance between carbon and iodine is not likely to be less than the value of 3·68 A. which is given by the co-ordinates now assigned to these atoms.

On this basis, the principal dimensions of the molecule are summarised in Table II.

TABLE II.

Dimensions of the 2 : 2′-di-iododiethyl trisulphide molecule.

Bond lengths, A.	Bond angles.
S–S = 2·04 ± 0·04	S–S–S = 113° ± 2°
C–S = 1·79	S–S–C = 120°
C–C = 1·54 (assumed)	S–C–C = 96°
C–I = 2·05	C–C–I = 100°

The shape of the molecule is interesting. It does, of course, possess an exact two-fold axis of symmetry in accordance with the space-group requirements. This axis is perpendicular to the (110) crystal plane and it passes through the central sulphur atom, S_2. Two views of the molecule are shown in Fig. 2, representing projections along the symmetry axis, and at right angles to that axis. The chain is almost fully extended but the atoms do not all lie in one plane. In fact, the plane of the central sulphur zigzag is nearly at right angles to the plane of the carbon–carbon–iodine zigzag. Starting from an original *trans*-planar configuration, the present model may be derived by making a very small rotation about the S_1S_2 bond, a larger rotation about the C_2S_1 bond, and finally a rotation of about 90° about the C_1C_2 bond.

Intermolecular Distances.—The measured intermolecular distances are shown in Fig. 3. The molecules are so packed that the iodine–iodine approach is never less than 4·4 A. The shortest iodine–sulphur intermolecular distance is 3·80 A., this being the distance from an iodine atom to the terminal sulphur atom of the S–S–S chain in the adjacent molecule. The distance from iodine to the central sulphur atom in the chain is 3·85 A.

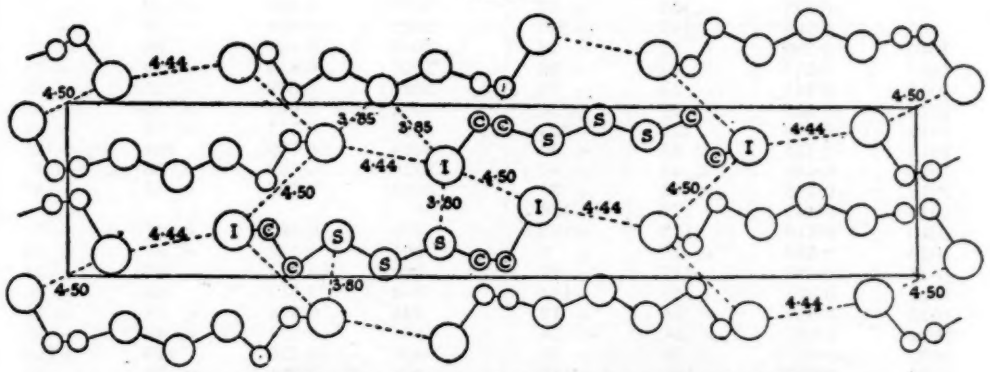
Discussion.

The sulphur–sulphur bond length found is shorter than that reported for orthorhombic sulphur (Warren and Burwell, *J. Chem. Physics*, 1935, **3**, 6) or by electron-diffraction study of sulphur vapour (Maxwell, Hendricks, and Moseley, *Physical Rev.*, 1936, **49**, 199; Lu and Donohue, *J. Amer. Chem. Soc.*, 1944, **66**, 818). These investigations favour the higher value of 2·12 A. The value of 2·04 A. has, however, been reported for dimethyl disulphide, studied by the electron-diffraction method by Stevenson and Beach (*ibid.*, 1938, **60**, 2872). We thus have both *X*-ray and electron-diffraction evidence confirming a decrease in bond length of about 0·08 A. in the polysulphides as compared with sulphur itself.

In the present structure the S–S–S bond angle of about 113° is somewhat larger than the values previously reported, which vary from 100° to about 106°.

The intermolecular distances found are in good agreement with those previously reported for sulphur and iodine. The observed iodine van der Waals radius of 2·22 A., when subtracted from the sulphur–iodine approach, gives a van der Waals radius for sulphur of 1·58 A. Warren and Burwell in their investigation of orthorhombic sulphur (*loc. cit.*) found a value of 1·65 A.

Fig. 3.



Intermolecular distances in the 2 : 2′-di-iododiethyl trisulphide structure.

Both these values are less than the sulphur ionic radius of 1·85 A., but the analogy between ionic and van der Waals radii is not likely to be so good for bivalent as for univalent ions (see also Pauling, " Nature of the Chemical Bond ", Cornell, 1939, p. 174).

Experimental.

Crystallisation.—Specimens of 2 : 2′-di-iododiethyl trisulphide were obtained from C.D.E.S., Porton. These were recrystallised from alcohol–benzene solution and yielded flat rectangular plates, m. p. 110°.

Intensity Measurements.—On account of high absorption ($\mu = 551$ per cm. for Cu-$K\alpha$ radiation) special precautions were necessary. It was found that the crystal specimens could readily be cut to uniform cross-sectional area. That used for intensity measurements was in the form of a lath, 0·13 mm. by 0·13 mm., and of length 0·65 mm., so that the necessity for absorption corrections was reduced for the ($h0l$) zone. The effect of the high absorption was further decreased by taking several series of films from crystals of slightly varying cross-sectional area and making a comparison of the intensities measured. Crystals were completely immersed in a beam of filtered copper radiation, and the reflections recorded photographically by means of a moving-film camera, using the multiple-film technique (Robertson, *J. Sci. Instr.*, 1943, **20**, 175). The absolute scale of the F values was not determined directly but was obtained by correlation with the calculated F values. The atomic scattering factor adopted for iodine was taken as a mean of the Thomas–Fermi scattering factors (" International Tables for the Determination of Crystal Structure ", p. 572) and the Pauling–Sherman scattering factors (*Z. Krist.*, 1932, **81**, 1). An arbitrary temperature correction was applied by means of the Debye–Waller temperature factor (" International Tables ", p. 570), using for the value of Θ the characteristic temperature given for crystalline iodine. The sulphur contribution was then weighted $\frac{1}{2}$ on this scale, and the carbon contribution was ignored in the evaluation of calculated structure factors. This rather empirical treatment was found to be sufficiently accurate for the determination of the trial structure, and in the final comparison of experimentally observed with calculated structure factors (Table III), the agreement is reasonably good.

Fourier Analysis.—The electron density on the *ac* plane (010) was computed at 900 points on the asymmetric unit. The *a* axis was subdivided into 30 parts and the *c* axis into 120 parts, the intervals along *a* being 0·200 A., and along *c* 0·245 A. The usual formula for $\rho(x, z)$ was employed, the summation being carried out by means of 3-figure strips of cosine factors and a mechanical sorting device (Robertson, *J. Sci. Instr.*, 1948, **25**, 216). This method is designed to give full 3-figure accuracy with

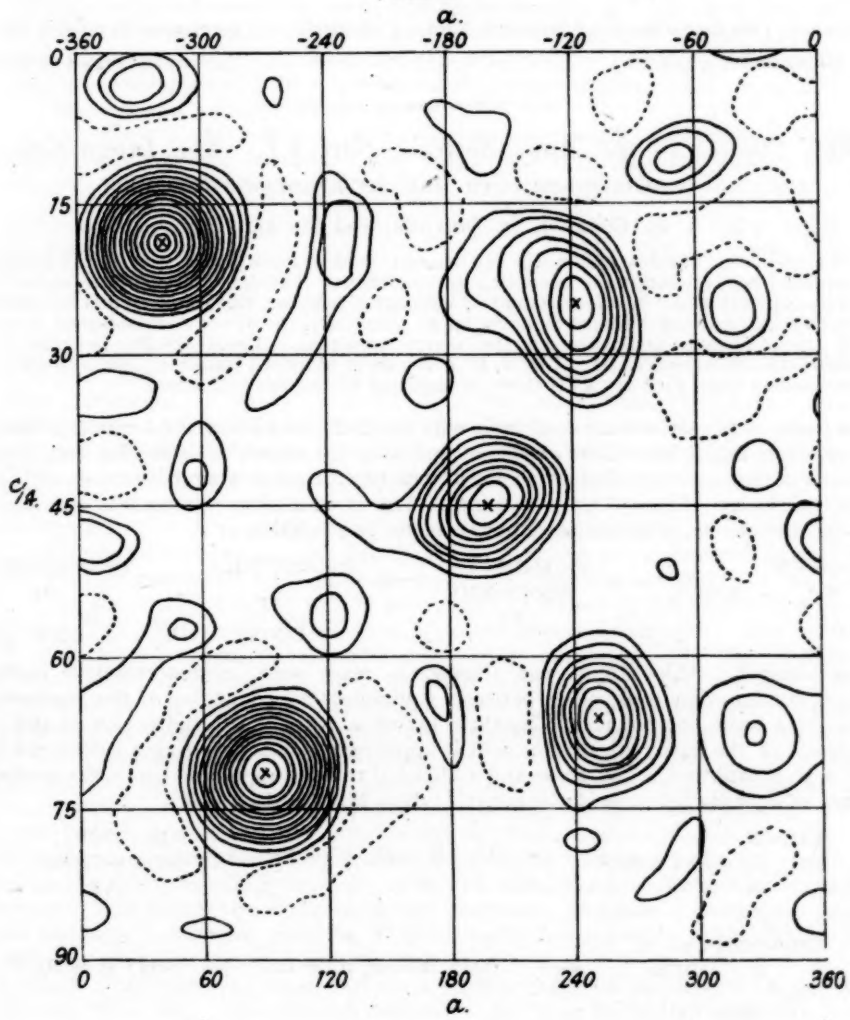
TABLE III.

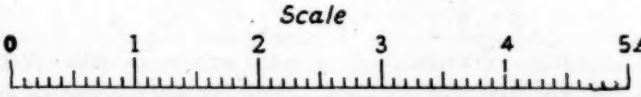
Measured and calculated values of the structure factor.

hkl.	$\sin\theta$ ($\lambda = 1{\cdot}54$).	F, meas.	F, calc.	*hkl.*	$\sin\theta$ ($\lambda = 1{\cdot}54$).	F, meas.	F, calc.
004	0·105	33	+ 29	301	0·385	57	− 46
008	0·211	427	−424	302	0·387	41	+ 24
0012	0·316	270	−275	303	0·392	82	− 63
0016	0·421	294	+282	304	0·398	270	−257
0020	0·525	184	+172	305	0·406	< 44	+ 31
0024	0·632	< 56	− 6	306	0·415	< 44	− 23
0028	0·738	139	−134	307	0·426	< 45	− 16
				308	0·438	156	−137
				309	0·451	< 46	0
101	0·130	81	+ 77	3010	0·465	46	+ 46
102	0·138	147	−124	3011	0·481	123	+121
103	0·150	81	+145	3012	0·497	171	+152
104	0·166	33	+ 64	3013	0·514	74	+ 46
105	0·184	81	+ 34	3014	0·532	74	− 34
106	0·203	270	−275	3015	0·551	< 50	0
107	0·224	107	−110	3016	0·570	196	+191
108	0·246	< 33	+ 32	3017	0·588	< 52	− 25
109	0·269	147	−150	3018	0·610	< 53	− 3
1010	0·293	41	− 28	3019	0·631	62	− 67
1011	0·317	74	− 96	3020	0·652	< 54	− 39
1012	0·341	41	− 36	3021	0·672	< 55	− 35
1013	0·365	57	− 24	3022	0·695	< 56	− 42
1014	0·390	229	+223	3023	0·717	< 56	− 41
1015	0·415	< 44	− 30	3024	0·739	163	−145
1016	0·440	< 45	− 30	3025	0·762	< 56	− 17
1017	0·466	< 46	+ 36	3026	0·785	< 55	− 47
1018	0·491	179	+156				
1019	0·516	115	+132	400	0·512	< 47	+ 22
1020	0·542	< 48	+ 9	401	0·512	< 47	− 24
1021	0·568	117	+ 97	402	0·514	246	−224
1022	0·594	156	−144	403	0·518	66	− 30
1023	0·620	< 55	+ 12	404	0·523	< 48	− 19
1024	0·645	< 56	+ 34	405	0·530	< 49	− 59
1025	0·671	< 56	− 32	406	0·536	115	+119
1026	0·697	148	−149	407	0·544	< 50	− 26
1027	0·723	< 57	− 45	408	0·551	< 50	+ 19
1028	0·749	< 57	+ 10	409	0·564	106	− 26
1029	0·775	< 56	− 43	4010	0·575	270	+230
1030	0·800	< 56	+ 39	4011	0·588	< 52	− 34
				4012	0·601	< 53	− 21
				4013	0·616	57	+ 85
200	0·256	205	−199	4014	0·631	< 53	0
201	0·258	131	− 89	4015	0·647	< 54	+ 19
202	0·261	197	+197	4016	0·663	< 54	+ 13
203	0·268	222	−165	4017	0·680	< 55	− 5
204	0·277	82	− 82	4018	0·698	180	−156
205	0·287	222	−181	4019	0·716	< 56	+ 13
206	0·301	82	− 97	4020	0·734	< 56	− 8
207	0·315	156	−141	4021	0·753	< 56	− 17
208	0·331	220	+245	4022	0·773	< 56	− 90
209	0·349	< 41	− 52	4023	0·793	< 55	− 27
2010	0·367	196	−200				
2011	0·386	90	+ 97	501	0·640	115	−117
2012	0·407	57	+ 33	502	0·642	< 53	− 25
2013	0·427	155	+112	503	0·645	< 54	− 11
2014	0·449	< 44	+ 1	504	0·649	98	+104
2015	0·471	164	+167	505	0·653	< 54	− 12
2016	0·493	< 45	− 67	506	0·659	82	− 60
2017	0·516	70	+ 82	507	0·666	82	+ 74
2018	0·539	131	+134	508	0·673	82	+ 69
2019	0·562	< 49	− 41	509	0·682	107	+ 98
2020	0·586	107	− 97	5010	0·692	58	+ 49
2021	0·610	73	− 23	5011	0·702	82	− 47
2022	0·634	82	+ 56	5012	0·714	56	− 64
2023	0·658	90	− 89	5013	0·725	< 56	+ 50
2024	0·682	< 55	+ 1	5014	0·738	< 56	+ 33
2025	0·707	91	− 92	5015	0·752	57	− 40
2026	0·731	91	− 67	5016	0·766	90	− 96
2027	0·756	82	− 20	5017	0·781	74	− 74
2028	0·781	< 56	+ 24	5018	0·799	< 56	+ 19

cell-side subdivision into 120 parts (3° intervals). The positions of the contour lines were obtained by graphical interpolation from the summation totals by making sections of the rows and columns. The resulting contour map is shown in Fig. 1, and an enlargement of a portion of it is given in Fig. 4.

FIG. 4.





One quarter of the unit cell, showing co-ordinates assigned to atoms. The zero-electron line is dotted. Each contour line represents an increase of 2 electrons per A.^{3}, except for the iodine atoms where the increment is 5 electrons per A.^{3}. The 2-electron line is omitted throughout.

Diffraction Effects.—It is clear from Fig. 4 that diffraction effects in the Fourier projection are of an order sufficient to preclude any observation of the carbon positions. The method of removing these effects adopted in the study of platinum phthalocyanine (Robertson and Woodward, *loc. cit.*) has not been attempted here because of the uncertain state of the sulphur and carbon scattering curves in compounds of this type, and the less accurate intensity estimations due to high absorption in the crystal. Spurious density maxima, rising to eight electrons per A.^{3}, are present near the iodine and the sulphur atom, but these effects are small compared with the iodine and sulphur peak values of about 70 and 20 electrons per A.^{3}, respectively.

Structure Factor Tables.—The co-ordinates of Table I and Fig. 4 were used for a final calculation of

the structure factors of all the planes. The results are collected in Table III under " F, calc." The agreement between the observed and calculated values of F, expressed in the usual way as a sum of all the discrepancies divided by the total of the measured structure factors, is 14·8% for the (*h*0*l*) reflections actually observed. If the absent reflections are included in the count, at their limiting, or " < " values, this figure is improved to 11·4%.

In conclusion we thank the Chief Scientist, Ministry of Supply, for permission to publish this paper.

THE UNIVERSITY, GLASGOW. [*Received, August 20th,* 1947.]

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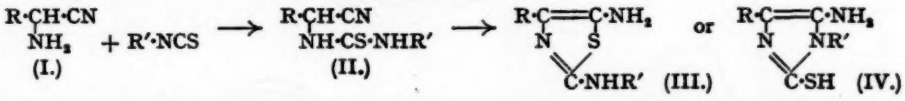
250. *Studies in the Azole Series. Part VI. The Interaction of α-Amino-nitriles and* iso*Thiocyanates.*

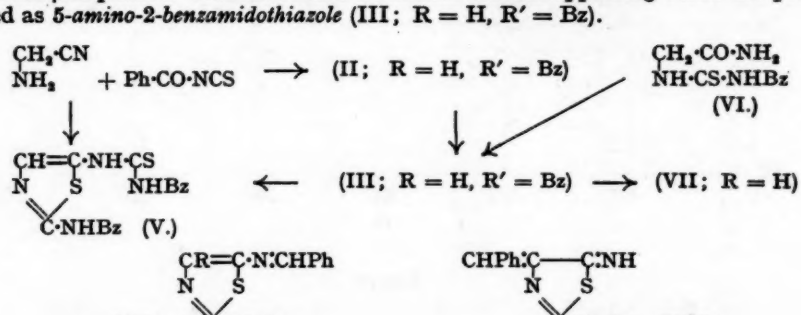
By A. H. COOK, J. D. DOWNER, and SIR IAN HEILBRON.

The interaction of aminoacetonitrile and benzoyl *iso*thiocyanate gives 5-amino- or 5-benzoylthioureido-2-benzamidothiazole according to conditions. The former rearranges under the influence of weak alkali into 5-benzamido-2-mercaptoglyoxaline, this isomerisation accounting for the varied products obtained on acetylation. Similarly, the use of α-aminobenzyl cyanide leads to 4-phenyl analogues of the above products. These results indicate that α-thioureido-nitriles such as (II; R = H, R′ = Bz) enjoy at most a transitory existence whereas corresponding ureas such as (XVI) show no tendency to undergo cyclisation.

EARLIER parts of this series were concerned with the facile formation of 5-aminothiazoles where acyclic products might have been expected, and with the object of extending such syntheses. The present communication deals with the interaction of some α-amino-nitriles with *iso*thiocyanates, particularly benzoyl *iso*thiocyanate. This combination of reactants was selected as it presented the chance of obtaining either thiazoles or glyoxalines :



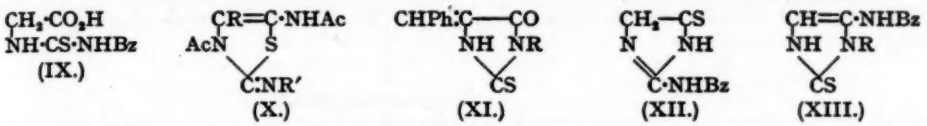
When benzoyl *iso*thiocyanate was allowed to react with aminoacetonitrile under mild conditions, combination took place between equimolecular quantities of the reactants with formation of a basic compound, $C_{10}H_9ON_3S$, giving a well-defined hydrochloride and *picrate*. On treatment of the base with nitrous acid it appeared to diazotise, giving a dark red dye on coupling with β-naphthol. For these and additional reasons appearing below the product was formulated as 5-*amino*-2-*benzamidothiazole* (III; R = H, R′ = Bz).



Convincing proof of the presence of a primary amino-group was provided by the formation of a Schiff's base, 2-*benzamido*-5-*benzylideneaminothiazole* (VII; R = H) with benzaldehyde. The remote possibility of this derivative being in fact a benzylidene derivative (VIII) of an imino-tautomeride of (III; R = H, R′ = Bz) was excluded by showing that substitution of the hydrogen atom in the 4-position in (III; R = H, R′ = Bz) in no way hindered condensation with benzaldehyde (see below). Again, the product (III; R = H, R′ = Bz) readily entered into further reaction with benzoyl *iso*thiocyanate to give a compound, $C_{18}H_{14}O_2N_4S_2$, which almost certainly is 2-*benzamido*-5-*benzoylthioureidothiazole* (V) (or a tautomeride thereof). The alternative thiourea (II; R = H, R′ = Bz) could hardly react in the above way, and indeed, it was shown that analogous substances failed to undergo any reaction with benzoyl *iso*thiocyanate under comparable conditions. Thus *N*-benzoyl-*N*′-methylthiourea was unaffected by benzoyl

*iso*thiocyanate, and incidentally by benzaldehyde also under the conditions used in forming the above Schiff's base. The compound (V) was also obtained directly from aminoacetonitrile sulphate in pyridine by reaction with benzoyl *iso*thiocyanate. In this connection *N'-benzoylthioureidoacetamide* (VI), prepared from aminoacetamide and benzoyl *iso*thiocyanate, was only weakly basic and underwent no further ready reaction with the latter reagent. Treatment with phosphorus tribromide, however, converted it into the *hydrobromide* of (III; R = H, R' = Bz) which also afforded the thioureido-derivative (V) by further reaction with benzoyl *iso*thiocyanate. Analogous was the conversion of the hydrochloride of (III; R = H, R' = Bz) into (V) by reaction with benzoyl *iso*thiocyanate.

The benzoyl group in (III; R = H, R' = Bz) was surprisingly firmly bound, vigorous acid hydrolysis of the compound bringing about ring-scission only with the formation of 2-thiohydantoin and *N*-benzoylthiohydantoic acid (IX) which was completely identified by its con-



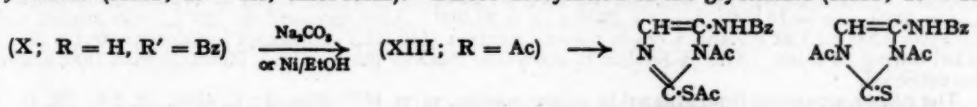
(IX.) (X.) (XI.) (XII.) (XIII.)

version into 3-benzoyl-5-benzylidene-2-thiohydantoin (XI; R = Bz) (Wheeler, Nicolet, and Johnson, *Amer. Chem. J.*, 1911, **46**, 468).

Whereas treatment of (III; R = H, R' = Bz) with benzoyl chloride gave 2 : 5-*dibenzamidothiazole*, acetylation gave more complex results. Excess of acetic anhydride gave a small yield of a diacetyl derivative, presumably (X; R = H, R' = Bz), together with a second product proved formally to be a monoacetyl derivative, though the following observations seem to establish that it was an acetyl derivative of a changed ring system.

The compound (III; R = H, R' = Bz) was devoid of pseudo-acidic properties, a fact clearly in keeping with its formulation and opposed to alternative cyclic structures such as (IV; R = H, R' = Bz). On being boiled with aqueous sodium carbonate, however, the thiazole (III; R = H, R' = Bz) was converted into a pseudo-acidic isomeride for which structures (IV; R = H, R' = Bz), (XII), and (XIII; R = H) came into consideration. The conversion of (III; R = H, R' = Bz) into (XII) would be entirely comparable with the isomerisation of 5-amino-2-mercaptothiazoles into dithiohydantoins (Parts II and III). On the other hand, the formation of (IV; R = H, R' = Bz) would represent an alternative still formally comparable with the known isomerisation mentioned above. Finally, the emergence of compound (XII; R = H) would represent simply a combination of the two changes already considered. Of these, the first can be excluded as the compound had no marked basic properties and failed to condense with benzaldehyde under conditions sufficing for the preparation of the Schiff's base (VII). Further, when treated with Raney nickel the new compound was very readily desulphurised, giving a substance which, though physically and chemically similar to 2-benzamidoglyoxaline (Fargher and Pyman, *J.*, 1919, **115**, 217), was not identical but isomeric with it. Structure (XII) is thus also excluded for the isomeride of 5-amino-2-benzamidothiazole and therefore the new compound must be 4(5)-*benzamido-2-mercaptoglyoxaline* (XIII; R = H), giving 4(5)-*benzamidoglyoxaline* by desulphurisation.

The above conclusion was supported by a further study of the results of acetylation of (III; R = H, R' = Bz). The diacetyl derivative (X; R = H, R' = Bz) was, as mentioned above, accompanied by a major quantity of a monoacetyl compound, $C_{12}H_{11}O_2N_3S$. The latter was also obtained by treating the diacetyl compound with sodium carbonate, and incidentally in abortive attempts to desulphurise it with Raney nickel in ethanol. Further acetylation of this monoacetyl derivative failed to regenerate the original diacetyl compound, an isomeric diacetyl derivative being obtained. It is probable therefore that the monoacetyl compound is not the thiazole but the acetyl derivative of (XIII; R = H). In view of its thiolic properties it is perhaps to be formulated as 4(5)-*benzamido-2-mercapto*-1- or -3-*acetylglyoxaline* (XIII; R = Ac, thiol form). Direct acetylation of the glyoxaline (XIII; R = H)



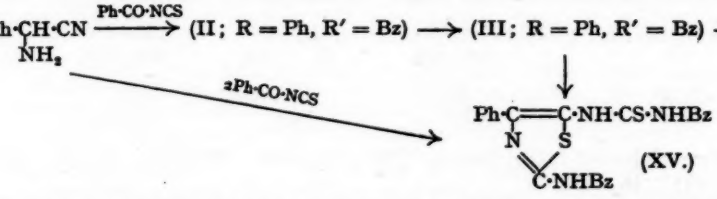
(X; R = H, R' = Bz) (XIII; R = Ac) (XIVa.) (XIVb.)

afforded a second monoacetyl derivative which, having regard to its origin and lack of pseudo-acidic properties, is tentatively regarded as 4(or 5)-*benzamido-2-acetylthioglyoxaline*, so that the

diacetyl compound mentioned above may be represented as 4(or 5)-*benzamido*-2-*acetylthio*-1- or -3-*acetylglyoxaline* (XIV*a*); it may be better represented by (XIV*b*), having regard to its inaccessibility from 4(or 5)-benzamido-2-acetylthioglyoxaline.

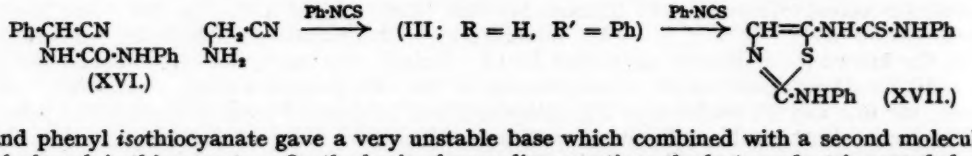
Finally, these considerations on the structure of compound (XIII) (and its derivatives) were confirmed by its hydrolysis to 2-thiohydantoin, identified as its 5-benzylidene derivative (XI; R = H) (Wheeler, Nicolet, and Johnson, *loc. cit.*).

Less extensive experiments employing α-aminobenzyl cyanide led to results which, as far as they were pursued, were very similar to those above. Reaction between equimolecular parts of the amino-nitrile and benzoyl *iso*thiocyanate in toluene led, not to the thiourea (II; R = Ph, R′ = Bz) which might have been expected, but to an isomeric base. The product is formulated as 5-*amino*-2-*benzamido*-4-*phenylthiazole* (III; R = Ph, R′ = Bz), for it was diazotisable and



afforded a *benzylidene* derivative (VII; R = Ph) as well as a *diacetyl* derivative, presumably (X; R = Ph, R′ = Bz). As with the analogous compound (III; R = H, R′ = Bz), the new thiazole (III; R = Ph, R′ = Bz) also reacted further with benzoyl *iso*thiocyanate, the product, 2-*benzamido*-5-*benzoylthioureido*-4-*phenylthiazole* (XV), being also obtainable directly from α-aminobenzyl cyanide.

The above facile cyclisations are, as expected, not observed in the corresponding oxygen series. For instance, ureidoacetonitrile, conveniently prepared from aminoacetonitrile and nitrourea (cf. Bailey, *Amer. Chem. J.*, 1897, **28**, 391), α-ureidobenzyl cyanide (Pinner and Lifschütz, *Ber.*, 1887, **20**, 2355; Pinner, *ibid.*, 1888, **21**, 2321), and *α-phenylureidobenzyl cyanide* (XVI) showed no tendency to pass into amino-oxazoles. On the other hand, aminoacetonitrile



and phenyl *iso*thiocyanate gave a very unstable base which combined with a second molecule of phenyl *iso*thiocyanate. On the basis of preceding reactions the last product is regarded as 2-*anilino*-5-*phenylureidothiazole* (XVII). Similarly, α-aminobenzyl cyanide and phenyl *iso*thiocyanate gave a stable base regarded as 5-*amino*-2-*anilino*-4-*phenylthiazole* (III; R = R′ = Ph) which formed a diacetyl derivative (X; R = R′ = Ph).

Experimental.

Lead thiocyanate (188 g.), benzoyl chloride (124 c.c.), and toluene (150 c.c.) were heated with stirring until reaction set in (*ca.* 80°), refluxed for 10 mins., and lead chloride separated from the cold solution and washed with toluene (30 c.c.). Fractionation of the solution in a vacuum gave benzoyl *iso*thiocyanate (104 g.), b. p. 135—138°/17 mm., which was redistilled and collected (yield, 93 g., 52%) at 95—97°/0·05 mm. (cf. Dixon, *J.*, 1899, **75**, 379; 1908, **93**, 692). The crude toluene solution could also be used for subsequent reactions.

Action of Benzoyl iso*Thiocyanate on Aminoacetonitrile.*—Benzoyl *iso*thiocyanate (12·2 c.c.) in ether (20 c.c.) was added during 15 mins. to aminoacetonitrile (Part III, this vol., p. 201) (5 g.) in ether (70 c.c.) with stirring at 0°. The oil solidified and after a further 10 mins. the colourless crystals (18 g.) were filtered off and washed with ether. 5-*Amino*-2-*benzamidothiazole* recrystallised from *ca.* 20 vols. of ethanol as colourless needles, m. p. 157° (decomp.) (yield, 13 g.) (Found: C, 54·8; H, 4·1; N, 18·8; S, 14·1. $C_{10}H_9ON_3S$ requires C, 54·8; H, 4·1; N, 19·2; S, 14·6%). Light absorption (chloroform): $\lambda_{max.}$ = 2820 A., ϵ = 16,400; $\lambda_{inflex.}$ = 2400 A., ϵ = 21,900. Aminoacetonitrile (20 g.) was stirred with dry ether (200 c.c.) at 0°, and a crude toluene solution (140 c.c.) of benzoyl *iso*thiocyanate (≡ 58 g.) added during 20 mins. After a further 15 mins. the thiazole (63 g., 81%), identical with that above, was collected.

The *picrate* separated from ethanol in yellow needles, m. p. 163° (Found: C, 43·0; H, 2·8; N, 18·7; S, 7·2. $C_{16}H_{12}O_8N_6S$ requires C, 42·8; H, 2·7; N, 18·7; S, 7·2%). Stirring the thiazole (2 g.) with 4% ethanolic hydrogen chloride (35 c.c.) gave immediately the corresponding hydrochloride, m. p. 200—201° (decomp.), which was diazotised with sodium nitrite, and also gave 2-benzamido-5-benzoylthioureidothiazole under the same conditions as did the corresponding hydrobromide (see below).

The preceding thiazole (1·5 g.) in pyridine (20 c.c.) containing benzoyl *iso*thiocyanate (1 c.c.) was brought to boiling. On cooling and diluting with methanol (30 c.c.), *2-benzamido-5-benzoylthioureidothiazole* (15 g.), m. p. 227°, separated. It separated from pyridine in lustrous green-yellow plates, m. p. 229° (decomp.) (Found: C, 56·4; H, 3·8; N, 14·8; S, 17·4. $C_{18}H_{14}O_2N_4S_2$ requires C, 56·5; H, 3·7; N, 14·7; S, 16·8%). Light absorption (in dioxan): $\lambda_{max.}$ = 2350, 2810, 3530 A., ϵ = 28,300, 29,800, 12,800, respectively; (in 0·1N-potassium hydroxide): $\lambda_{max.}$ = 2280 A., ϵ = 9550. This compound was also prepared as follows: Aminoacetonitrile sulphate (17 g.) was suspended in pyridine (60 c.c.), and a solution of benzoyl *iso*thiocyanate (27 g.) in toluene (200 c.c.) added while the mixture was stirred and slowly heated to boiling under reflux. An exothermic reaction set in and pyridine sulphate separated as a gum with some yellow crystals. Decanting and washing the deposit with ethanol (300 c.c.) removed the pyridine salt to leave the compound (5 g.) previously obtained, m. p. 228—229° (decomp.). 2-Benzamido-5-benzoylthioureidothiazole (1 g.) in 1N-sodium hydroxide (8 c.c.) was shaken with methyl sulphate. After 30 mins., dilution with water (15 c.c.) and neutralisation gave the *dimethyl* derivative (1 g.) as a yellow solid which after two recrystallisations from ethanol had m. p. 180° (decomp.) (Found: C, 58·6; H, 4·5; N, 13·4; S, 15·1. $C_{20}H_{18}O_2N_4S_2$ requires C, 58·5; H, 4·4; N, 13·7; S, 15·6%). Light absorption (chloroform): $\lambda_{max.}$ = 2490, 2810, 3340 A., ϵ = 25,400, 20,900, 22,150, respectively.

Aminoacetamide (10 g.) in ethanol (100 c.c.) and water (12 c.c.) was stirred at ~ 0° while benzoyl *iso*thiocyanate (18·3 c.c.) was added during 5 mins. After a further 15 mins. the solid (11 g.) was filtered off and dried. *N'-Benzoylthioureidoacetamide* recrystallised from ethanol in lustrous silky needles, m. p. 209—210° (slight decomp.) (Found: C, 50·9; H, 4·9; N, 17·6; S, 13·2. $C_{10}H_{11}O_2N_3S$ requires C, 50·6; H, 4·7; N, 17·7; S, 13·5%); light absorption (dioxan): $\lambda_{max.}$ = 2390, 2810 A., ϵ = 23,250, 9950. The preceding compound (3 g.) in hot dioxan (50 c.c.) was treated with phosphorus tribromide (0·5 c.c.). The copious cream precipitate (3·8 g.) was filtered off and washed with ether. *5-Amino-2-benzamidothiazole hydrobromide* had m. p. 198° (decomp.) and decomposed on attempted crystallisation (Found: N, 14·2. $C_{10}H_{10}ON_3SBr$ requires N, 14·0%). The hydrobromide gave a red solution with aqueous nitrous acid which afforded a deep red precipitate on addition to alkaline β-naphthol. The hydrobromide (1 g.) in pyridine (5 c.c.) was treated with benzoyl *iso*thiocyanate (0·5 c.c.) in pyridine (2 c.c.), and the mixture heated to boiling, cooled, and poured into methanol (25 c.c.). 2-Benzamido-5-benzoylthioureidothiazole (0·4 g.), m. p. and mixed m. p. with the material above 229°, was precipitated.

Miscellaneous Reactions of 5-Amino-2-Benzamidothiazole.—5-Amino-2-benzamidothiazole (5 g.) was refluxed with acetic anhydride (20 c.c.) for 15 mins. and the cold solution stirred into ice-water (250 c.c.) for 30 mins. The crude product (6·5 g., m. p. 124—127°) was crystallised repeatedly from ethanol to give *5-acetamido-2-benzimido-3-acetylthiazoline* as white lustrous plates, m. p. 185° (Found: C, 55·6; H, 4·4; N, 13·6; S, 10·4. $C_{14}H_{13}O_3N_3S$ requires C, 55·4; H, 4·3; N, 13·9; S, 10·6%). Light absorption (chloroform): $\lambda_{max.}$ = 2860 A., ϵ = 13,650; (0·01N-potassium hydroxide): $\lambda_{max.}$ = 2280, 3360 A., ϵ = 14,550, 14,550. The mother-liquors from the above diacetyl derivative were diluted with water, and the solid obtained crystallised repeatedly from methanol to give hydrated crystals of *4(5)-benzamido-2-mercapto-1- or 3-acetylglyoxaline* (?) (1·7 g.) in colourless needles, m. p. 249—250°. When this was heated in a vacuum for 5 hours at 140°, the anhydrous substance was obtained as a white amorphous powder, m. p. 251° (Found: C, 55·0; H, 4·1; N, 15·8; S, 12·0. $C_{12}H_{11}O_2N_3S$ requires C, 55·1; H, 4·2; N, 16·1; S, 12·3%). Light absorption (ethanol): $\lambda_{max.}$ = 2290, 3150 A., ϵ = 13,050, 12,400; (0·1N-potassium hydroxide): $\lambda_{max.}$ = 2250, 3470 A., ϵ = 15,150, 12,800. The latter compound was also obtained by boiling the preceding diacetyl derivative (1·5 g.) with 10% aqueous sodium carbonate (20 c.c.) for 10 mins. and collecting the solid (1·35 g.). It was further obtained by boiling the same diacetyl derivative (1 g.) with Raney nickel (4—6 g.) in ethanol (20 c.c.).

4-Benzamido-2-mercapto-3-acetylglyoxaline (0·8 g.), refluxed with acetic anhydride (6 c.c.) for 10 mins., changed to a mass of green-yellow silky needles without melting. Pouring into water gave *4-benzamido-2-acetylthio-3-acetyliminazole* (?) (0·7 g.) which recrystallised from a large volume of acetic anhydride in needles, m. p. 199° (Found: C, 55·8; H, 4·5; N, 13·9; S, 10·1. $C_{14}H_{13}O_3N_3S$ requires C, 55·4; H, 4·3; N, 13·9; S, 10·6%). Light absorption (chloroform): $\lambda_{max.}$ = 3150 A., ϵ = 12,100; (in 0·1N-potassium hydroxide): $\lambda_{max.}$ = 2270, 3470 A., ϵ = 17,400, 14,550. The second acetyl group is very labile, crystallisation of the compound from pyridine giving almost entirely the original monoacetyl derivative.

5-Amino-2-benzamidothiazole (3 g.) was refluxed in benzene (50 c.c.) with benzoyl chloride (2 c.c.) for 90 mins. On cooling, the derivative (2·6 g.) was collected and recrystallised from nitrobenzene–ethanol, hydrated 2 : 5-*dibenzamidothiazole* separating; m. p. 295° (decomp.) (Found, after drying at 56°: C, 59·5; H, 4·2; N, 12·1; S, 8·7. $C_{17}H_{13}O_2N_3S$ requires C, 59·8; H, 4·4; N, 12·3; S, 9·4%). Light absorption (ethanol): $\lambda_{max.}$ = 2280, 3240 A., ϵ = 22,850, 17,050. The monohydrate was dried in a vacuum at 140° to constant weight (54·9 Mg. lost 3·3 mg. of water. Calc. for 1 mol.: 3·15 mg.).

5-Amino-2-benzamidothiazole (2 g.) was refluxed with benzaldehyde (1 c.c.) in ethanol (40 c.c.) for 30 mins., the Schiff's base quickly separating. The *benzylidene* derivative (2·7 g.) crystallised from ethanol in yellow needles, m. p. 233° (Found: C, 66·7; H, 4·4; N, 13·5; S, 10·7. $C_{17}H_{13}ON_3S$ requires C, 66·4; H, 4·3; N, 13·7; S, 10·4%). Light absorption (chloroform): $\lambda_{max.}$ = 2350, 3050, 3660 A., ϵ = 23,000, 8000, 31,950, respectively.

5-Amino-2-benzamidothiazole (4 g.) was refluxed for 3 hrs. with ethanol (120 c.c.) while hydrogen chloride was passed in, then cooled and filtered; the filtrate was evaporated to dryness, and the residue taken up in water (30 c.c.) and neutralised with aqueous sodium carbonate. Ethyl ω-benzoylthiohydantoate was precipitated and recrystallised from ethanol in lustrous flakes, m. p. 130° (Wheeler, Nicolet, and Johnson, *loc. cit.*, quote m. p. 128—129°) (Found: S, 12·1. Calc. for $C_{12}H_{14}O_2N_2S$: S, 12·0%).

5-Amino-2-benzamidothiazole (5 g.) was refluxed for 40 mins. with concentrated hydrochloric acid (50 c.c.). The product was poured into ethanol (150 c.c.) and filtered hot. The solid, *N*-benzoylthiohydantoic acid, crystallised from dilute ethanol, had m. p. 199° (Wheeler, Nicolet, and Johnson give m. p. 202°). The preceding compound (0·25 g.), sodium acetate (0·3 g.), acetic acid (2·5 c.c.), acetic anhydride (1 c.c.), and benzaldehyde (0·25 c.c.) were heated together to 100—140° for 50 mins., and the

mixture poured into water (100 c.c.). The solid crystallised from ethanol as yellowish-red plates of 3-benzoyl-5-benzylidenethiohydantoin, m. p. 181° (lit., m. p. 181°). The filtrate from the above hydrolysis of 5-amino-2-benzamidothiazole on cooling gave 2-thiohydantoin, which separated from water in yellow crystals, m. p. 225—227° (decomp.) undepressed on mixing with an authentic specimen, m. p. 228° (decomp.). The benzylidene derivative of 2-thiohydantoin had m. p. 257—258°, also undepressed by authentic material, m. p. 258° (Wheeler, Nicolet, and Johnson, *loc. cit.*).

5-Amino-2-benzamidothiazole (5 g.) was refluxed for 5 mins. with 10% aqueous sodium carbonate (60 c.c.). On cooling, light brown crystals (4·3 g.) separated. 4(5)-*Benzamido-2-mercaptoglyoxaline* crystallised from ethanol in fine, colourless, hairy needles, decomposing at 240°, m. p. rather indefinite at 260° (Found: C, 54·5; H, 4·2; N, 19·5; S, 14·5. $C_{10}H_9ON_3S$ requires C, 54·8; H, 4·1; N, 19·2; S, 14·6%). Light absorption (ethanol): $\lambda_{max.}$ = 2670, 2280, 3050 A., ϵ = 17,100, 17,100, 6800, respectively. The compound coupled with sodium diazobenzene-*p*-sulphonate to give a carmine dye. On boiling the glyoxaline (1·5 g.) for 45 mins. with concentrated hydrochloric acid (10 c.c.) and then adding ethanol (25 c.c.), 2-thiohydantoin (0·5 g.) was precipitated. It crystallised from water in yellow needles, m. p. 226° (decomp.), identical with an authentic specimen. It was also identified as its benzylidene derivative, which crystallised from dilute acetic acid with m. p. 255—257° undepressed by the authentic material (Wheeler, Nicolet, and Johnson, *loc. cit.*).

4(or 5)-Benzamido-2-mercaptoglyoxaline (12 g.) was stirred in ethanol (400 c.c.) under reflux with Raney nickel (24 g.) for 1 hour. On filtration and concentration of the filtrate 4(or 5)-*benzamidoglyoxaline* (6 g.) separated in fine, colourless, hairy needles, m. p. 217° (slight decomp.), unchanged on recrystallisation from ethanol (Found: C, 64·2; H, 4·9; N, 22·3. $C_{10}H_9ON_3$ requires C, 64·1; H, 4·8; N, 22·5%). Light absorption (chloroform): $\lambda_{max.}$ = 2270 A., ϵ = 9350. A mixture with authentic 2-benzamidoiminazole gave m. p. *ca.* 200°.

4(5-)Benzamido-2-mercaptoglyoxaline (1 g.) was refluxed with acetic anhydride (10 c.c.) for 30 mins., and the cold solution poured into water (70 c.c.). The precipitate (0·6 g.) was recrystallised from ethanol to give *5-benzamido-2-acetylthioiminazole* (?) as almost colourless needles, m. p. 204° (Found: C, 55·0; H, 4·3; S, 11·7. $C_{12}H_{11}O_2N_3S$ requires C, 55·1; H, 4·2; S, 12·3%).

Action of iso*Thiocyanates on α-Aminobenzyl Cyanide.*—A solution of α-aminobenzyl cyanide (15 g.) in toluene (230 c.c.) containing benzoyl *iso*thiocyanate (20 g.) was warmed slightly and then kept for 3 days. The yellow solid (22 g.) recrystallised from *n*-butanol to give *5-amino-2-benzamido-4-phenylthiazole* as small, yellow, silky plates, m. p. 179° (Found: C, 65·4; H, 4·6; S, 10·5. $C_{16}H_{13}ON_3S$ requires C, 65·1; H, 4·4; S, 10·8%). Light absorption (in chloroform): $\lambda_{max.}$ = 3090 A., ϵ = 12,100; (in 0·1N-potassium hydroxide): $\lambda_{max.}$ = 2310, 3100 A., ϵ = 21,550, 4425. The compound was diazotised in acetic–sulphuric acid, the diazonium salt coupling with β-naphthol to give a red dye.

The preceding thiazole was boiled in pyridine with excess of benzoyl *iso*thiocyanate for 1 min., and the solution cooled and poured into ethanol. The product was *2-benzamido-5-benzoylthioureido-4-phenylthiazole*, m. p. 203°, which was more conveniently prepared as follows: α-Aminobenzyl cyanide (5 g.) was kept overnight in ether (50 c.c.) with benzoyl *iso*thiocyanate (10 c.c.). The yellow deposit (6·7 g., m. p. 202°) crystallised from ethanol in pale yellow hairs of the monohydrate, m. p. 205° (Found: C, 60·3; H, 4·3; N, 11·9; S, 13·3. $C_{24}H_{20}O_3N_4S_2$ requires C, 60·5; H, 4·2; N, 11·8; S, 13·5%). Light absorption (in chloroform): $\lambda_{max.}$ = 2440, 2820, 3760 A., ϵ = 43,800, 26,200, 9,300, respectively; (in 0·1N-potassium hydroxide): $\lambda_{max.}$ = 3380 A., ϵ = 12,400. The monohydrate was dried in a vacuum at 140° to constant weight (47·8 Mg. lost 2·1 mg. Calc. for 1 mol.: 1·9 mg.).

5-Amino-2-benzamido-4-phenylthiazole (1 g.) was refluxed for 2 hrs. with acetic anhydride (10 c.c.), and the solution cooled and poured into ice-water (150 c.c.). Recrystallisation of the solid from ethanol gave *5-acetamido-2-benzimido-3-acetyl-4-phenylthiazoline* (0·7 g.) as colourless needles, m. p. 180° (Found: C, 63·3; H, 4·8; N, 11·0; S, 8·7. $C_{20}H_{17}O_3N_4S$ requires C, 63·3; H, 4·5; N, 11·1; S, 8·5%). Light absorption (chloroform): $\lambda_{max.}$ = 2390, 2810, 2040 A., ϵ = 35,250, 17,450, 13,300, respectively.

5-Amino-2-benzamido-4-phenylthiazole (1·5 g.) was refluxed for 30 mins. in ethanol (30 c.c.) with benzaldehyde (0·5 c.c.), and the fine yellow needles (1·8 g.) collected. Recrystallisation from ethanol gave the *benzylidene* derivative as yellow needles, m. p. 197—198° (Found: C, 72·6; H, 4·7; N, 10·9. $C_{23}H_{17}ON_3S$ requires C, 72·1; H, 4·5; N, 11·0%). Light absorption (chloroform): $\lambda_{max.}$ = 2420, 2590, 3070, 3950 A., ϵ = 31,000, 31,000, 14,550, 25,650, respectively.

Phenyl *iso*cyanate (5·2 c.c.) was slowly added to α-aminobenzyl cyanide (5 g.) in ether (100 c.c.); heat was evolved and a bulky precipitate (7 g.) appeared. Recrystallisation from ethanol gave *α-phenylureidobenzyl cyanide* as white hairs, m. p. 155° (Found: C, 72·0; H, 5·2; N, 16·5. $C_{15}H_{13}ON_3$ requires C, 71·7; H, 5·2; N, 16·7%).

Nitrourea (10 g.), suspended in water (20 c.c.), was treated with aminoacetonitrile (5 g.) in water (10 c.c.). On warming the clear solution to 60—70° and allowing it to cool and the effervescence to subside, long needles of ureidoacetonitrile (6 g., m. p. 136—137°) separated; a further yield (2·7 g.) of slightly impure material was obtained from the mother-liquor. The urea, crystallised from ethanol, had m. p. 138° (Bailey, *Amer. Chem. J.*, 1897, **28**, 391, quotes m. p. 139°) (Found: N, 42·4. Calc. for $C_3H_5ON_3$: N, 42·4%) (cf. Davis, *J. Amer. Chem. Soc.*, 1929, **51**, 1790).

Phenyl *iso*thiocyanate (7 c.c.) was added to α-aminobenzyl cyanide (8 g.), and the mixture heated to boiling. After cooling, the crystalline solid was collected (3 g., m. p. 198—199°) and recrystallised from ethanol to give colourless needles of *5-amino-2-anilino-4-phenylthiazole*, m. p. 199° (Found: C, 67·4; H, 4·9; N, 15·6; S, 12·4. $C_{15}H_{13}N_3S$ requires C, 67·4; H, 4·9; N, 15·7; S, 12·0%). Light absorption (ethanol): $\lambda_{max.}$ = 3130 A., ϵ = 15,100; (0·1N-potassium hydroxide): $\lambda_{max.}$ = 2290, 3080 A., ϵ = 18,700, 6250.

5-Amino-2-anilino-4-phenylthiazole (1 g.) was refluxed for 1 hour with acetic anhydride (10 c.c.), and the solution cooled and poured into water (50 c.c.). Recrystallisation of the solid from ethanol gave colourless needles of *5-acetamido-2-phenylimino-3-acetyl-4-phenylthiazoline*, m. p. 245° (Found: C, 64·5; H, 5·0; N, 11·9; S, 9·2. $C_{19}H_{17}O_2N_3S$ requires C, 64·9; H, 4·9; N, 12·0; S, 9·1%). Light absorption (in chloroform): $\lambda_{max.}$ = 2950 A., ϵ = 18,250; (in 0·1N-potassium hydroxide): $\lambda_{infl.}$ = 2900 A., ϵ = 11,600.

Phenyl *iso*thiocyanate (20 c.c.) in ether (25 c.c.) was slowly added to aminoacetonitrile (10 g.) with shaking. The solid (31·5 g.), m. p. 146—147°, presumably 5-amino-2-anilinothiazole, could not be crystallised satisfactorily. A portion (2·3 g.) was boiled in pyridine (5 c.c.) with phenyl *iso*thiocyanate (2 c.c.) for 1 min., and the solution poured into methanol (100 c.c.). The solid (2 g., m. p. 218—219°) was recrystallised from pyridine–ethanol, giving *2-anilino-5-phenylthioureidothiazole* as yellow microcrystals, m. p. 221° (Found: C, 58·4; H, 4·3; S, 19·8. $C_{16}H_{14}N_4S_2$ requires C, 58·8; H, 4·3; S, 19·7%). Light absorption (chloroform): $\lambda_{max.}$ = 3020, 3870 A., ϵ = 12,050, 28,700.

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251. *Phenylpropiolic Acids. Part II. The Dimerisation of p-Nitro- and p-Methoxy-phenylpropiolic Acid.*

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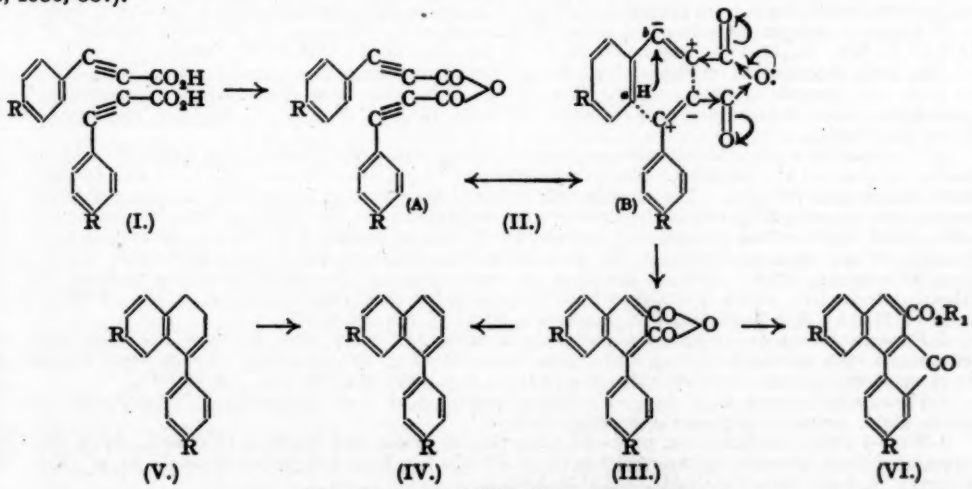
p-Nitrophenylpropiolic acid (I; R = NO_2) is converted by acetic anhydride into *4′ : 7-dinitro-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride* (III; R = NO_2). The dibasic *acid* from this is decarboxylated to *4′ : 7-dinitro-1-phenylnaphthalene* (IV; R = NO_2). This is reduced to 4′ : 7-diamino-1-phenylnaphthalene, which is deaminated to 1-phenylnaphthalene.

p-Methoxyphenylpropiolic acid (I; R = OMe) gives, when similarly treated, the analogous *anhydride* (III; R = OMe), and thence the *acid* and *4′ : 7-dimethoxy-1-phenylnaphthalene* (IV; R = OMe). This is found to be identical with a specimen prepared by dehydrogenation of *4′ : 7-dimethoxy-1-phenyl-3 : 4-dihydronaphthalene* (V; R = OMe). The dimerisation is believed to be preceded by anhydride formation.

IN Part I (Baddar, *J.*, 1947, 224) it was shown that *o*-methoxyphenylpropiolic acid was converted by the action of acetic anhydride or acetyl chloride into 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride. In the present investigation, the authors have studied the effect of substituents on the mode of dimerisation of phenylpropiolic acids.

p-Nitrophenylpropiolic acid (I; R = NO_2) was converted by boiling acetic anhydride into *4′ : 7-dinitro-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride* (III; R = NO_2). Its structure was determined by decarboxylating its dibasic *acid* to *4′ : 7-dinitro-1-phenylnaphthalene* (IV; R = NO_2), which was reduced to *4′ : 7-diamino-1-phenylnaphthalene*. This gave on deamination 1-phenylnaphthalene, identified as its mononitro-derivative.

p-Methoxyphenylpropiolic acid (I; R = OMe) similarly gave the *anhydride* (III; R = OMe), the dibasic *acid* from which was decarboxylated to *4′ : 7-dimethoxy-1-phenylnaphthalene* (IV; R = OMe), identical with a specimen prepared by dehydrogenation of *4′ : 7-dimethoxy-1-phenyl-3 : 4-dihydronaphthalene* (V; R = OMe), which was prepared from 7-methoxy-α-tetralone and *p*-methoxyphenylmagnesium bromide (cf. Howell and Robertson, *J.*, 1936, 587).



(R = H, NO_2 or OMe; R_1 = H or Me)

4′ : 7-Dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride was converted, under the influence of aluminium chloride in nitrobenzene, into 3′ : 7-*dimethoxy*-3 : 4-*benzfluorenone*-1-*carboxylic acid* (VI; R = OMe; R_1 = H) (cf. Part I, *loc. cit.*). Under similar conditions, the nitro-derivative (III; R = NO_2) was recovered unchanged, and resinified on heating.

The fact that unsubstituted and substituted phenylpropiolic acids (see Part I, *loc. cit.*, for references) gave only 1-phenylnaphthalene derivatives, independently of the nature of the substituent, may be explained by assuming that dimerisation is preceded by anhydride formation [II(A); R = H, OMe, or NO_2]. The hydrogen attached to carbon atom (*a*) migrates as a proton to carbon atom (*b*), then the excited structure II(B) cyclises as indicated by the dotted lines.

This is supported by the following facts: (1) All the reagents used for converting phenylpropiolic acids into 1-phenylnaphthalene-2 : 3-dicarboxylic anhydrides, such as acetic anhydride, acetyl chloride, or phosphorus oxychloride, are anhydride-forming reagents. (2) Phenylpropiolic anhydride, prepared by treating an ethereal solution of propiolyl chloride with silver propiolate, dimerised readily when heated on the water-bath, exposed to sun-light, or even kept at room temperature, forming 1-phenylnaphthalene-2 : 3-dicarboxylic anhydride. Contrary to Bucher (*J. Amer. Chem. Soc.*, 1908, **30**, 1264; footnote), phenylpropiolic acids were recovered unchanged under these conditions.

Experimental.

(M. p.s are not corrected. Microanalyses were carried out by Drs. Weiler and Strauss, Oxford).

p-*Nitrophenylpropiolic Acid* (I; R = NO_2).—The finely powdered phenylpropiolic acid (10 g.) was added portionwise to fuming nitric acid (100 c.c.; *d* 1·52), which had been cooled to − 18°, and the mixture stirred for one hour (cf. Reich, *Compt. rend.*, 1916, **162**, 129). The product was crystallised from 60% ethyl alcohol, from which *p*-nitrophenylpropiolic acid was obtained in prismatic needles, m. p. 201—202°; yield, 7 g. (54%). Müller (*Annalen*, 1882, **212**, 139) and Drewsen (*ibid.*, p. 154) give m. p.s 181° and 198°, respectively. It gave on oxidation with alkaline potassium permanganate *p*-nitrobenzoic acid.

4′ : 7-*Dinitro*-1-*phenylnaphthalene*-2 : 3-*dicarboxylic Anhydride* (III; R = NO_2).—*p*-Nitrophenylpropiolic acid (4 g.) was refluxed with acetic anhydride (18 c.c.) for 2 hours, cooled, and the crystalline precipitate filtered off. On crystallisation from glacial acetic acid, 4′ : 7-*dinitro*-1-*phenylnaphthalene*-2 : 3-*dicarboxylic anhydride* was obtained in reddish-brown crystals, m. p. 325—326° (Found: C, 59·2; H, 2·1; N, 8·2. $C_{18}H_8O_7N_2$ requires C, 59·3; H, 2·2; N, 7·7%); yield, 65%. The *acid*, prepared by refluxing the anhydride with dilute sodium hydroxide solution, crystallised from glacial acetic acid in pale yellowish-brown crystals, m. p. above 320° (Found: C, 56·6; H, 2·7; N, 6·9; equiv., 192·7. $C_{18}H_{10}O_8N_2$ requires C, 56·5; H, 2·6; N, 7·3%; equiv., 191).

The *diethyl* ester, prepared by refluxing the acid (2 g.) with absolute ethyl alcohol (20 c.c.), and concentrated sulphuric acid (5 c.c.) for 3 hours, crystallised from ethyl alcohol in light yellowish-brown flakes, m. p. 177—178° [Found: C, 60·1; H, 4·1; N, 6·3; *M* (Rast), 430. $C_{22}H_{18}O_8N_2$ requires C, 60·3; H, 4·1; N, 6·4%; *M*, 438]; yield, 70%.

4′ : 7-*Dinitro*-1-*phenylnaphthalene* (IV; R = NO_2).—4′ : 7-Dinitro-1-phenylnaphthalene-2 : 3-dicarboxylic acid (2·0 g.) was dissolved in hot quinoline (15 c.c.), and heated to 210—220°. Copper-bronze (0·5 g.) was then added, and the mixture stirred for 45 minutes. The reaction mixture was poured into cold dilute hydrochloric acid, and the precipitated product filtered off, and repeatedly extracted with boiling benzene. The benzene extract was washed with sodium hydroxide solution, and the product crystallised from glacial acetic acid or benzene–light petroleum (b. p. 60—70°), from which 4′ : 7-*dinitro*-1-*phenylnaphthalene* was obtained in orange-red crystals, m. p. 201—202° (Found: C, 65·3; H, 3·4; N, 9·6. $C_{18}H_{10}O_4N_2$ requires C, 65·3; H, 3·4; N, 9·5%); yield, 1·2 g. (78%).

The acid, precipitated on the acidification of the alkaline extract, crystallised from 80% acetic acid to give rod-shaped crystals, m. p. 260—261°. This was most probably 4′ : 7-*dinitro*-1-*phenylnaphthalene*-2- or -3-*carboxylic acid* (Found: C, 60·2; H, 3·3; N, 7·9. $C_{17}H_{10}O_6N_2$ requires C, 60·3; H, 3·0; N, 8·3%).

4′ : 7-*Diamino*-1-*phenylnaphthalene*.—Zinc dust (20 g.) was added portionwise during 30 minutes to a boiling mixture of 4′ : 7-dinitro-1-phenylnaphthalene (1·0 g.), ethyl alcohol (120 c.c.), and concentrated hydrochloric acid (80 c.c.). The mixture was refluxed for a further hour, with occasional shaking, and poured into ice-cold 30% sodium hydroxide solution (400 c.c.). The liberated base was extracted with ether, dried, and the base precipitated as its hydrochloride by passing a dry current of hydrogen chloride through the dry ethereal solution. The base (0·6 g.) was liberated with sodium hydroxide, but changed to a dark-brown, sticky solid on standing, so was converted (Schotten–Baumann method) into its *dibenzoyl* derivative, which crystallised from benzene in fine pale pink crystals, m. p. 249—250° (Found: C, 80·7; H, 5·1; N, 6·7. $C_{30}H_{22}O_2N_2$ requires C, 81·4; H, 5·0; N, 6·3%).

1-*Phenylnaphthalene*.—1-Phenylnaphthalene-2′-carboxylic acid (0·4 g.) was decarboxylated by refluxing it with quinoline (4 c.c.), and copper-bronze (0·14 g.) for 1·5 hours. The product was worked up as usual, and purified by distillation in a vacuum, b. p. 180—200°/10 mm.; yield, 90%.

1-Phenylnaphthalene-2 : 3- and -2′ : 3-dicarboxylic acid were similarly decarboxylated, but in poorer yield, owing to incomplete decarboxylation.

4-Nitro-1-phenylnaphthalene, prepared according to Weiss and Woidich (*Monatsh.*, 1925, **46**, 453), crystallised from benzene–light petroleum (b. p. 60—70°) in brownish-yellow crystals, m. p. 131—132° (Found: N, 5·6. Calc. for $C_{16}H_{11}O_2N$: N, 5·6%).

Deamination of 4′ : 7-*Diamino*-1-*phenylnaphthalene*.—The mixture of the finely powdered

4′ : 7-diamino-1-phenylnaphthalene (0·5 g.) and concentrated sulphuric acid (3 c.c.) was cooled in a freezing mixture, with stirring. A solution of sodium nitrite (0·5 g.) in glacial acetic acid (2 c.c.) was slowly added during 15 mins., followed by glacial acetic acid (2 c.c.) added dropwise during 15 mins. The stirring was continued for a further 30 mins., and then the diazonium solution was slowly added to a vigorously stirred suspension of finely powdered, freshly prepared cuprous oxide (0·65 g.) in alcohol (10 c.c.). The reaction was accompanied by evolution of heat, but the temperature was not allowed to exceed 70°. The stirring was continued for an hour, then the reaction mixture was poured into water (100 c.c.), and the precipitated product filtered off. Both the precipitate and the filtrate were extracted with benzene, and the product distilled in a vacuum to give a yellowish-brown viscous oil (0·2 g.), b. p. 180—200°/10 mm. This was identified as 1-phenylnaphthalene by converting it into its 4-nitro-derivative, m. p. 131—132°, undepressed on admixture with an authentic specimen (Found: N, 5·9. Calc. for $C_{16}H_{11}O_2N$: N, 5·6%).

Methyl p-*Methoxycinnamate*.—This was obtained in 95% yield from the acid chloride and methyl alcohol; m. p. 94—95°. Perkin (*J.*, 1881, **39**, 439) gives m. p. 89°.

p-*Methoxyphenylpropiolic Acid* (I; R = OMe).—Powdered pure methyl $\alpha\beta$-dibromo-β-(*p*-methoxyphenyl)propionate (40 g.), m. p. 150—151°, was slowly added to cold 20% alcoholic potassium hydroxide (150 c.c.), the mixture heated under reflux for 10 hours, and worked up as usual (cf. Part I, *loc. cit.*). The product was contaminated with an acid containing bromine, and was therefore purified by repeated fractional crystallisation from benzene, from which *p*-methoxyphenylpropiolic acid was obtained in colourless prismatic needles, m. p. 142—143° (Found: C, 68·1; H, 4·7; OMe, 15·2. Calc. for $C_{10}H_8O_3$: C, 68·2; H, 4·6; OMe, 17·6%); yield, 9 g. (45%). Reychler (*Bull. Soc. chim.*, 1897, **17**, 512) gives m. p. 132—139°.

4′ : 7-*Dimethoxy*-1-*phenylnaphthalene*-2 : 3-*dicarboxylic Anhydride* (III; R = OMe).—*p*-Methoxyphenylpropiolic acid (8·5 g.) was refluxed with acetic anhydride (25 c.c.) for 4 hours, cooled, and the precipitated anhydride filtered off (cf. Part I, *loc. cit.*). On crystallisation from glacial acetic acid the *anhydride* was obtained in straw-yellow, tabular, prismatic crystals, m. p. 216—217° [Found: C, 71·9; H, 4·45; OMe, 17·3; *M* (Rast), 345. $C_{20}H_{14}O_5$ requires C, 71·8; H, 4·2; OMe, 18·6%; *M*, 334]; yield, 6·6 g. (82%). This anhydride (0·5 g.) was refluxed with 20% sodium hydroxide solution (15 c.c.), filtered, cooled in ice, and acidified. The precipitated 4′ : 7-*dimethoxy*-1-*phenylnaphthalene*-2 : 3-*dicarboxylic acid* crystallised from glacial acetic acid in colourless crystals, m. p. 239—240° (Found: C, 67·6; H, 4·8. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·6%). Its *dimethyl* ester, prepared in dioxan solution by means of diazomethane in ether, crystallised from benzene–light petroleum (b. p. 60—70°) in colourless crystals, m. p. 126—127° (Found: C, 69·6; H, 5·2. $C_{22}H_{20}O_6$ requires C, 69·5; H, 5·3%).

Methyl 3′ : 7-*Dimethoxy*-3 : 4-*benzfluorenone*-1-*carboxylate* (VI; R = OMe; R_1 = Me).—The powdered mixture of the anhydride (III; R = OMe) (3 g.) and aluminium chloride (15 g.) was slowly added to ice-cold nitrobenzene (36 c.c.), and the reaction was carried out as described in Part I (*loc. cit.*). The dry crude acid (2·9 g.), being difficultly soluble in most organic solvents, was directly esterified by allowing its suspension in dioxan (25 c.c.) to stand at room temperature (20—30°) for 3 days with ethereal diazomethane. The precipitated dark-brown product was filtered off, and washed with hot ammonium hydroxide. On crystallisation from dioxan–ethyl alcohol, *methyl* 3′ : 7-*dimethoxy*-3 : 4-*benzfluorenone*-1-*carboxylate* separated in red prismatic needles, m. p. 191—192° (Found: C, 72·1; H, 4·5. $C_{21}H_{16}O_5$ requires C, 72·4; H, 4·6%). Hydrolysis of this ester with alcoholic potassium hydroxide afforded the pure acid.

4′ : 7-*Dimethoxy*-1-*phenyl*-3 : 4-*dihydronaphthalene* (V; R = OMe).—Ice-cold *p*-methoxyphenylmagnesium bromide, prepared from *p*-bromoanisole (3·3 g.; 1·2 mols.) and magnesium metal (0·45 g.; 1·3 atoms) in dry ether (25 c.c.), was treated dropwise with a solution of 7-methoxy-α-tetralone (2·5 g., 1 mol.) in 25 c.c. of ether during ½ hour. The reaction mixture was left over-night at room temperature, and then refluxed for 2 hours. The product was hydrolysed with ice-cold ammonium chloride solution, subjected to steam distillation to remove unchanged materials, and finally purified by vacuum distillation. The fraction of b. p. 195—205°/4 mm. was crystallised from methyl alcohol, from which 4′ : 7-*dimethoxy*-1-*phenyl*-3 : 4-*dihydronaphthalene* separated in colourless tabular prismatic crystals, m. p. 77—78° (Found: C, 80·9; H, 6·8; OMe, 20·8. $C_{18}H_{18}O_2$ requires C, 81·2; H, 6·8; OMe, 23·3%); yield, 1·4 g. (37%).

4′ : 7-*Dimethoxy*-1-*phenylnaphthalene* (IV; R = OMe).—A mixture of 4′ : 7-dimethoxy-1-phenyl-3 : 4-dihydronaphthalene (0·2 g.) and powdered selenium (0·2 g.) was heated at 280—285° for 15 hours (cf. Howell and Robertson, *loc. cit.*). The product was extracted with ether, and then purified by crystallisation from methyl alcohol, from which 4′ : 7-*dimethoxy*-1-*phenylnaphthalene* was obtained in colourless monoclinic crystals, m. p. 87—88° (Found: C, 81·8; H, 6·2. $C_{18}H_{16}O_2$ requires C, 81·8; H, 6·1%).

Decarboxylation of 4′ : 7-*Dimethoxy*-1-*phenylnaphthalene*-2 : 3-*dicarboxylic Acid*.—This was carried out in an exactly similar manner to that described for 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic acid (Part I, *loc. cit.*). The product was distilled in a vacuum, b. p. 200—210°/5 mm., then crystallised from methyl alcohol, from which 4′ : 7-dimethoxy-1-phenylnaphthalene was obtained in colourless crystals, m. p. 87—88°, undepressed on admixture with an authentic specimen (Found: C, 81·2; H, 6·2. Calc. for $C_{18}H_{16}O_2$: C, 81·8; H, 6·1%); yield, 44%.

Phenylpropiolic Anhydride (II; R = H).—An ethereal solution of phenylpropiolyl chloride (Ruhemann and Merriman, *J.*, 1905, **87**, 139) was mixed with powdered silver phenylpropiolate (Glaser, *Annalen*, 1870, **154**, 148) and kept over-night at 15—20° with occasional shaking. The ethereal solution was filtered, and the ether removed at room temperature, under reduced pressure, to give phenylpropiolic anhydride as a light-brown viscous oil. It could not be purified by distillation, as it was converted on heating into 1-phenylnaphthalene-2 : 3-dicarboxylic anhydride. The precipitated silver chloride was found to be contaminated with the anhydride (III; R = H).

Conversion of Phenylpropiolic Anhydride into 1-*Phenylnaphthalene*-2 : 3-*dicarboxylic Anhydride*.—This conversion was effected by either (*a*) heating phenylpropiolic anhydride on the water-bath for

15—20 mins., or (*b*) exposing its benzene solution (trace of iodine) to sun-light for several days. In both cases 1-phenylnaphthalene-2 : 3-dicarboxylic anhydride was obtained as a crystalline product, m. p. 255—256°. Partial conversion was also effected at room temperature. Under similar conditions, phenylpropiolyl chloride was recovered unchanged.

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252. 1-*Phenylnaphthalenes. Part I. The Synthesis of* 2′ : 5-*Dimethoxy*-1-*phenyl*-, 4′ : 7-*Dimethoxy*-1-*phenyl*-, *and* 1-p-*Tolyl*-7-*methyl-naphthalene*-2 : 3-*dicarboxylic Acids.*

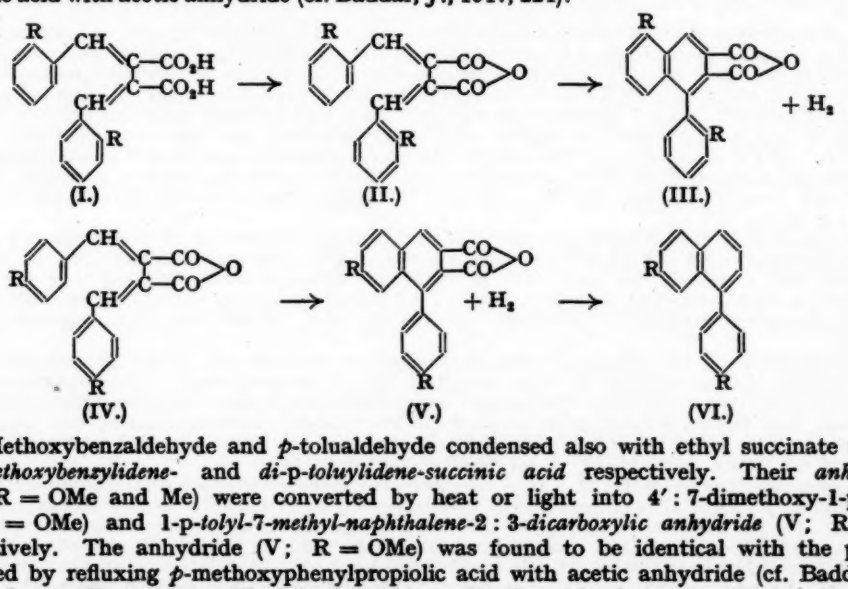
By F. G. BADDAR, LANSON S. EL-ASSAL, and M. GINDY.

o- and *p*-Methoxybenzaldehyde and *p*-tolualdehyde condense with ethyl succinate to give *di*-o-*methoxybenzylidene*- (I; R = OMe), *di*-p-*methoxybenzylidene*-, and *di*-p-*toluylidene-succinic acid* respectively. The *anhydrides* (II; R = OMe; IV, R = OMe and Me) are converted by the action of heat or light into the corresponding 1-phenylnaphthalene derivatives (III; R = OMe; and V; R = OMe and Me respectively).

THE condensation of benzaldehyde with ethyl succinate in presence of sodium ethoxide was reported by Stobbe and Kloeppel (*Ber.*, 1894, **27**, 2405), Stobbe and Naoúm (*Ber.*, 1904, **37**, 2241), and Stobbe, Ljungren, and Freyberg (*Ber.*, 1926, **59**, 270) to give dibenzylidenesuccinic acid (I; R = H). The anhydride (II; R = H) was converted into 1-phenylnaphthalene-2 : 3-dicarboxylic anhydride (III; R = H) on exposure to sunlight (Stobbe, *Ber.*, 1907, **40**, 3372). On distillation with barium hydroxide the dibasic acid (I; R = H) was converted into 1-phenylnaphthalene (Stobbe, *loc. cit.*), owing to cyclisation and decarboxylation.

In the present investigation, the authors repeated the experiments of Stobbe, Ljungren, and Freyberg (*loc. cit.*), but were unable to reproduce their results (35—40% yield). After several trials, using sodium or potassium, or their ethoxides, both in the cold and in the hot, we were able to find the favourable conditions for raising the yield to 15—20% only.

o-Methoxybenzaldehyde was similarly condensed with ethyl succinate to give *di*-o-*methoxybenzylidenesuccinic acid* (I; R = OMe). The *anhydride* (II; R = OMe) was converted by heat or light into 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride (III; R = OMe), which was identical with the product obtained by heating *o*-methoxyphenylpropiolic acid with acetic anhydride (cf. Baddar, *J.*, 1947, 224).



p-Methoxybenzaldehyde and *p*-tolualdehyde condensed also with ethyl succinate to give *di*-p-*methoxybenzylidene*- and *di*-p-*toluylidene-succinic acid* respectively. Their *anhydrides* (IV; R = OMe and Me) were converted by heat or light into 4′ : 7-dimethoxy-1-phenyl- (V; R = OMe) and 1-p-*tolyl*-7-*methyl-naphthalene*-2 : 3-*dicarboxylic anhydride* (V; R = Me) respectively. The anhydride (V; R = OMe) was found to be identical with the product obtained by refluxing *p*-methoxyphenylpropiolic acid with acetic anhydride (cf. Baddar and El-Assal, preceding paper). The dibasic *acid* from (V; R = Me), however, was decarboxylated to 1-*p*-tolyl-7-methylnaphthalene (VI; R = Me), which was found to be identical with a specimen prepared by the dehydrogenation of 1-*p*-tolyl-7-methyl-3 : 4-dihydronaphthalene,

obtained by the action of 7-methyl-α-tetralone on *p*-tolylmagnesium iodide (cf. Baddar and El-Assal, *loc. cit.*).

The conversion of the dibenzylidene derivatives (II; R = H and OMe; and IV; R = OMe and Me) into the corresponding 1-phenylnaphthalenes (III; R = H and OMe; and V; R = OMe and Me respectively) appears to proceed by the elimination of hydrogen, followed by the activation of the molecule and cyclisation, as stated by Baddar and El-Assal (*loc. cit.*).

Experimental.

(M. p.s are not corrected. Microanalyses were carried out by Drs. Weiler and Strauss, Oxford.)

Di-o-methoxybenzylidenesuccinic Acid (I; R = OMe).—A suspension of freshly prepared, powdered, alcohol-free sodium ethoxide (6·4 g.; 2 mols.) in dry ether (70 c.c.) was cooled to — 18°, while thoroughly stirred. A solution of ethyl succinate (8·3 g.; 1 mol.) and *o*-methoxybenzaldehyde (13·0 g.; 2 mols.) in dry ether (30 c.c.) was quickly added, and the mixture stirred for 12 hours, the temperature being kept between — 14° and — 10°. The temperature was then raised to 0°, and the mixture kept for 3 days, with occasional stirring. The product was then decomposed with water, the ether distilled off, and the aqueous alkaline layer refluxed for 1 hour, to hydrolyse any ester present. The solution was extracted with ether to remove neutral substances, and the precipitated acids were taken up in ether, and re-extracted from the ethereal solution with dilute potassium hydroxide solution (charcoal). The product, precipitated on acidification, was purified by extracting it with boiling water two or three times. The insoluble portion was repeatedly crystallised from glacial acetic acid, from which *di-o-methoxybenzylidenesuccinic acid* was obtained in colourless monoclinic crystals, m. p. 259—260° (decomp.). On slow heating, it began to melt at 200°, probably owing to anhydride formation [Found: C, 67·9; H, 5·2; *M* (Rast), 358. $C_{20}H_{18}O_6$ requires C, 67·8; H, 5·1%; *M*, 354]; yield, 3·6 g. (*ca.* 20%). It gave with concentrated sulphuric acid a yellow solution with a faint green fluorescence.

Dibenzylidenesuccinic Acid (I; R = H).—This was similarly prepared as mentioned above in a 15—20% yield. The acid was purified, however, by precipitating it as its barium salt (cf. Stobbe and Naoúm, *loc. cit.*).

Di-o-methoxybenzylidenesuccinic Anhydride (II; R = OMe).—The acid (I; R = OMe) (4·5 g.) was refluxed with acetyl chloride (30 c.c.) for 1—2 hours. The solution was concentrated to about one-third of its original volume, then ether was added, and the mixture left overnight. The orange-yellow crystalline precipitate was collected and crystallised from benzene, from which *di-o-methoxybenzylidenesuccinic anhydride* separated in orange-yellow crystals, m. p. 195·5—196·5° (efferv.) (Found: C, 71·2; H, 4·9. $C_{20}H_{16}O_5$ requires C, 71·4; H, 4·8%); yield, 4·1 g. (98%). It dissolved in concentrated sulphuric acid to a yellow solution.

2′ : 5-Dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic Anhydride (III; R = OMe).—This was prepared from the anhydride (II; R = OMe) in a nearly quantitative yield, by either heating it at 280—285° for 2 hours, or exposing its benzene or chloroform solution, containing a trace of iodine, to sunlight for two days (March). The product was crystallised from glacial acetic acid, from which 2′ : 5-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride separated in straw-yellow, tabular, prismatic crystals, m. p. 245—246° (Found: C, 71·8; H, 4·3; OMe, 18·2. Calc. for $C_{20}H_{14}O_5$: C, 71·8; H, 4·2; OMe, 18·6%). The m. p. was not depressed on admixture with the product obtained by heating *o*-methoxyphenylpropiolic acid with acetic anhydride (cf. Baddar, *loc. cit.*).

Di-p-methoxybenzylidenesuccinic Acid.—A suspension of powdered, freshly prepared, alcohol-free sodium ethoxide (6·8 g.; 2 mols.) in dry ether (70 c.c.) was mechanically stirred, while cooled in a freezing mixture to between — 18° and — 15°. A solution of *p*-methoxybenzaldehyde (13·6 g.; 2 mols.) and ethyl succinate (8·8 g.; 1 mol.) in dry ether (30 c.c.) was quickly added, and the mixture kept at this temperature for 6 hours. The temperature was then raised to 0°, kept thereat for 2 days, and then finally at room temperature (30—35°) for another day, with occasional stirring. The product was worked up as for the *o*-methoxy-derivative, and then extracted with hot benzene to remove the soluble resinous acids. The insoluble product was crystallised from glacial acetic acid, from which *di-p-methoxybenzylidenesuccinic acid* separated in colourless crystals, m. p. 260—261° (quick heating) (Found: C, 67·7; H, 5·0. $C_{20}H_{18}O_6$ requires C, 67·8; H, 5·1%).

Di-p-methoxybenzylidenesuccinic Anhydride (IV; R = OMe).—The powdered acid (0·2 g.) was refluxed with acetyl chloride (4 c.c.) for 4 hours, then the acetyl chloride was removed, and ether added. The ethereal solution was left overnight, and the crystalline precipitate was collected, and crystallised from benzene–light petroleum (b. p. 55—65°), from which *di-p-methoxybenzylidenesuccinic anhydride* separated in orange-yellow flakes, m. p. 179—180° [Found: C, 71·6; H, 4·7; *M* (Rast), 319. $C_{20}H_{16}O_5$ requires C, 71·4; H, 4·8%; *M*, 336]; yield, 90%.

4′ : 7-Dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic Anhydride (V; R = OMe).—This was obtained from the anhydride (IV; R = OMe) by either heating it at 205—210° for 2 hours, or exposing its benzene or chloroform solution, containing traces of iodine, to sun-light for 4 days (July). In both cases, the product was crystallised from glacial acetic acid or benzene–light petroleum (b. p. 60—70°) to give 4′ : 7-dimethoxy-1-phenylnaphthalene-2 : 3-dicarboxylic anhydride in straw-yellow, tabular, prismatic crystals, m. p. 216—217°, undepressed when mixed with a specimen obtained by refluxing *p*-methoxyphenylpropiolic acid with acetic anhydride (cf. Baddar and El-Assal, *loc. cit.*) (Found: C, 71·6; H, 4·4; OMe, 17·5. Calc. for $C_{20}H_{14}O_5$: C, 71·8; H, 4·2; OMe, 18·6%).

Di-p-toluylidenesuccinic Acid.—A suspension of powdered, alcohol-free sodium ethoxide (6·8 g.; 2 mols.) in dry ether (80 c.c.) was cooled to between — 18° and — 15°, while thoroughly stirred. A solution of *p*-tolualdehyde (12·0 g.; 2 mols.) and ethyl succinate (8·8 g.; 1 mol.) in dry ether (30 c.c.) was quickly added, and the reaction carried out as in the case of di-*p*-methoxybenzylidenesuccinic acid. The dry acid was extracted with a little hot benzene, and the insoluble product was crystallised from 70% acetic acid, from which *di-p-toluylidenesuccinic acid* separated in light yellowish-brown crystals, m. p.

219—220° (quick heating), and 217—218° (slow heating), probably owing to anhydride formation (Found: C, 73·9; H, 5·8. $C_{20}H_{16}O_4$ requires C, 74·5; H, 5·6%); yield, 1·8 g. (20%).

Di-p-toluylidenesuccinic Anhydride (IV; R = Me).—This was similarly prepared by refluxing the acid (0·5 g.) with acetyl chloride (2 c.c.) for 1 hour. On crystallisation from benzene–light petroleum (b. p. 55—65°) *di-p-toluylidenesuccinic anhydride* was obtained in yellow prismatic crystals, m. p. 177—178° (Found: C, 78·9; H, 5·1. $C_{20}H_{16}O_3$ requires C, 78·9; H, 5·3%); yield, 95%.

1-p-*Tolyl-7-methylnaphthalene-2*: 3-*dicarboxylic Anhydride* (V; R = Me).—This was similarly prepared by either heating di-*p*-toluylidenesuccinic anhydride at 205—210° for one hour, or exposing its benzene solution to sunlight for 8 days (March). On crystallisation from benzene–light petroleum (b. p. 55—65°) 1-p-*tolyl-7-methylnaphthalene-2*: 3-*dicarboxylic anhydride* was obtained in colourless crystals, m. p. 266—267° [Found: C, 79·0; H, 4·6; *M* (Rast), 297. $C_{20}H_{14}O_3$ requires C, 79·4; H, 4·7%; *M*, 302].

1-p-*Tolyl-7-methylnaphthalene-2*: 3-*dicarboxylic Acid*.—The anhydride (V; R = Me) was boiled with dilute sodium hydroxide solution for 10 minutes. The acid precipitated on acidification was crystallised from benzene, from which 1-p-*tolyl-7-methylnaphthalene-2*: 3-*dicarboxylic acid* separated in colourless fine needles, m. p. 270—271° (Found: C, 74·5; H, 5·3. $C_{20}H_{16}O_4$ requires C, 75·0; H, 5·0%).

1-p-*Tolyl-7-methylnaphthalene* (VI; R = Me).—1-*p*-Tolyl-7-methyl-3: 4-dihydronaphthalene was prepared from 7-methyl-α-tetralone (3·6 g.) and *p*-tolylmagnesium iodide (from 5·4 g. of *p*-iodotoluene) in ether (50 c.c.). The reaction was carried out as in the case of the 4′: 7-dimethoxy-derivative (cf. Baddar and El-Assal, *loc. cit.*), and the product was obtained as a pale-yellow viscous oil, b. p. 175—180°/4 mm., which failed to crystallise.

Dehydrogenation of this compound (0·7 g.) with powdered selenium (0·6 g.) at 280—285° for 15 hours gave an ether-soluble product, from which 1-*p*-tolyl-7-methylnaphthalene was obtained as a pale-yellow fluorescent viscous oil, b. p. 170—180°/2 mm. (0·2 g.), which failed to solidify. Nitration of this substance (0·36 g.) in acetic acid (6 c.c.) with nitric acid (1·5 c.c.; *d* 1·42) (cf. Howell and Robertson, *J.*, 1936, 587) at room temperature, in the course of 4 hours, gave a *mononitro*-derivative, which separated from light petroleum (b. p. 55—65°) in yellowish-brown crystals, m. p. 94—95° (Found: C, 77·5; H, 5·7; N, 5·0. $C_{18}H_{15}O_2N$ requires C, 78·0; H, 5·5; N, 5·1%).

Decarboxylation of 1-p-*Tolyl-7-methylnaphthalene-2*: 3-*dicarboxylic Acid*.—The acid was decarboxylated in a similar manner to that adopted for the 4′: 7-dimethoxy-derivative (cf. preceding paper). The product was purified by distillation in a vacuum to give 1-*p*-tolyl-7-methylnaphthalene as a yellowish-brown fluorescent oil, b. p. 170—180°/2 mm. (yield, 60%). The product was nitrated, in an exactly similar manner to that mentioned above, to give a mononitro-derivative, m. p. 94—95°, undepressed when mixed with an authentic specimen (Found: C, 77·5; H, 5·5; N, 4·9. Calc. for $C_{18}H_{15}O_2N$: C, 78·0; H, 5·5; N, 5·1%).

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253. *The Fractionation of Cerium*(III) *and Neodymium Mixtures on an Ion-exchange Column.*

By B. A. LISTER and M. L. SMITH.

The possibility of separating simple mixtures of cerium and neodymium salts by preferential ion exchange has been examined. A novel multitube column has been designed and in use has achieved the partial separation of these ions, cerium being taken up preferentially by the exchanger. A decrease in the total concentration of the mixed solution has resulted in increased separation, which may be due to a difference in the relative degree of hydration of the two ion species at different concentrations.

WALTON (*J. Franklin Inst.*, 1941, **232**, 305) has reviewed the published data on the equilibria between cations of the same valency in the alkali and the alkaline-earth series on various exchangers, and has shown that for each of these series the higher the atomic weight the more firmly was the ion retained. The experiments of Jenny (*Kolloid-Beih.*, 1927, **23**, 428) appeared especially conclusive in these two series, but in his experimental arrangements the salt concentration was continuously varying.

Since with increasing ionic radius there is a decrease in hydration in solution, it also appeared that ion retention increased as the hydration was lower. This latter correspondence was supported by the experiments of Wiegner (*J. Soc. Chem. Ind.*, 1931, **50**, 65T; *Z. Pflanz., Düngung, Bodenk.*, 1929, **14**, *A*, 321) who showed that in a range of alcohol–water mixtures the displacing powers of $Cs^{\cdot}$ and $Na^{\cdot}$ became more nearly alike as the proportion of alcohol was increased, *i.e.*, as the hydration differences fell.

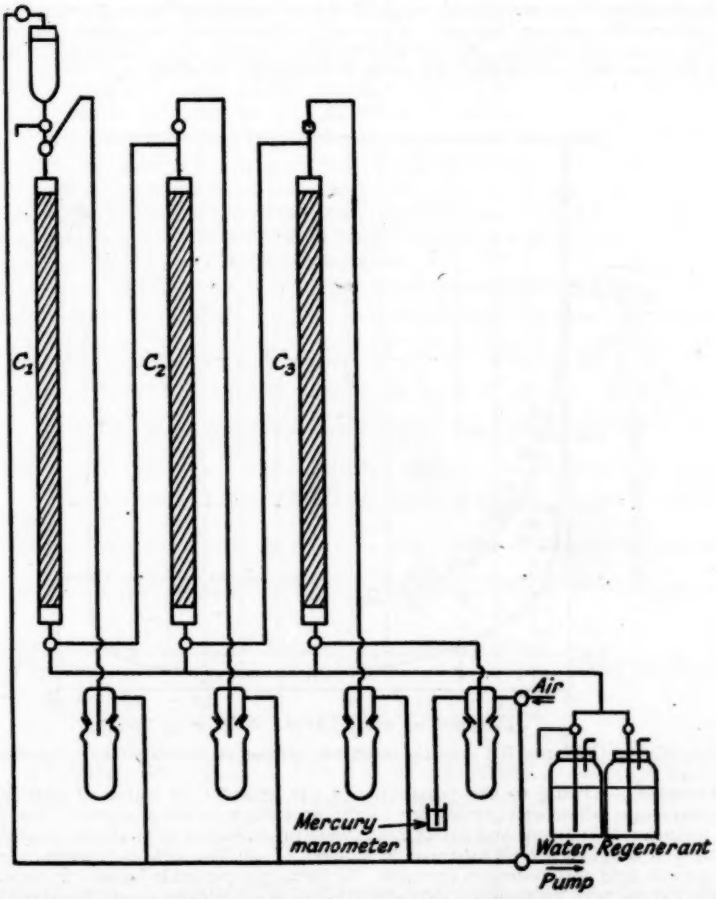
However, Russell and Pearce (*J. Amer. Chem. Soc.*, 1943, **65**, 595), in some preliminary experiments on the separation of a complex mixture of rare-earth group ions on a 50-ft. zeolite column, have reported that the ions of larger radius (*e.g.*, La, 1·22 A., Nd, 1·15 A.) are held less firmly than the smaller ions (Y, 1·06 A.) and consequently appear first in the percolate from the

base of the column. This conclusion is confirmed in a general way by the work of Kozak and Walton (*J. Physical Chem.*, 1945, **49**, 471).

We were interested in the separation of members of the rare-earth series by ion exchange, and in order to assess the possibilities, carried out some preliminary experiments with solutions of cerous, praseodymium, and neodymium salts (ionic radii : Ce 1·18 A., Pr 1·16 A., Nd 1·15 A.).

Preliminary Experiments on Exchangers.—The following exchangers were available (*a*) " Basex ", a synthetic zeolite similar to " Crystallite ". (*b*) " Decalso ", a synthetic siliceous gel. (*c*) " Zeokarb Na ", a sulphonated coal. (*d*) " Zeokarb H.I.M. ", a sulphonated coal. (*e*) " Zeokarb H.I.P. ", a sulphonated coal. The first three were Na^+-ion, and the last two H^+-ion exchangers.

FIG. 1.



Line diagram of the multitube column apparatus (C_3 represents 9 columns in series).

A series of up-take and regeneration experiments were carried out with solutions of cerous nitrate on each of these exchangers, using both the static and the column technique. The results of these tests showed that (*e*) had the most rapid and highest up-take and the most satisfactory regeneration properties. This exchanger was used in all subsequent work.

Static up-take tests to compare the exchange characteristics of Ce^{***}, Pr^{***}, and Nd^{***} on Zeokarb H.I.P. showed that the exchange characteristics of the three ions are so similar that any differences lie within the limits of sensitivity of the experimental and analytical methods and are therefore not evident.

EXPERIMENTAL.

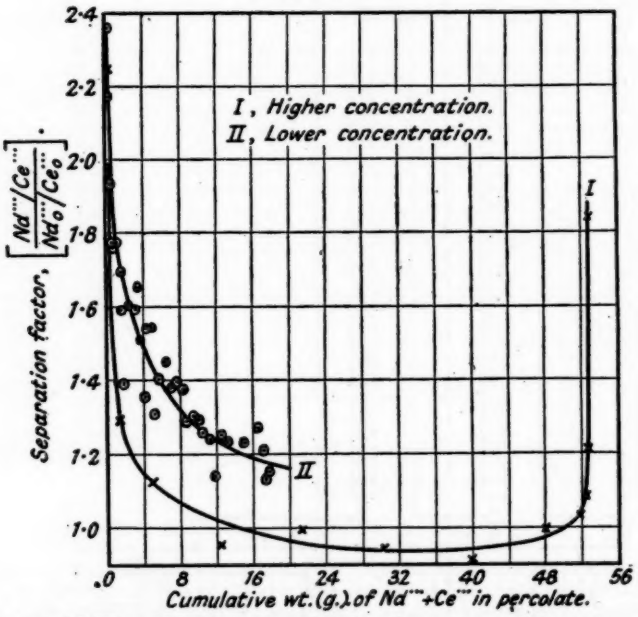
Apparatus.—Preliminary work showed that a long column was essential if any significant separation of cerium-group ions was to be achieved, and clearly a column approximating to the 50-ft. length used by Russell and Pearce was indicated. For convenience, and to avoid the difficulty of support, a multitube

model was designed and constructed consisting of eleven columns, each 110 cm. long and 1 cm. in diameter, giving a total length of 12 m. Fig. 1 is a line diagram of the apparatus. In this series construction, to ensure even flow, a departure was made from the normal procedure in that before addition of the mixture the apparatus was completely filled with pure solvent, in this case water. This initial filling was made (from a reservoir attached to the base of each column) by application of suction at the head, together with suitable manipulation of the stop-cocks at each end of the separate limbs.

In the runs with mixtures of cerous and neodymium nitrates the rare-earth solution was allowed to percolate slowly through the apparatus from a reservoir at the head of the initial column. Slight suction was necessary to ensure a reasonable flow rate, a rate of 1·4 ml./min. being maintained with a reduction in pressure of 150 mm. of mercury. Each 10-ml. fraction of the percolate was tested for the presence of rare-earth ions with (*a*) saturated oxalic acid solution and (*b*) alcoholic quinalizarin and sodium hydroxide solutions. When rare-earth ions were detected, the nitrate solution in the reservoir was replaced by water, and 10 ml. fractions of percolate were collected until rare-earth ions were no longer detectable.

For the regeneration of the Zeokarb, two procedures were possible with our apparatus: (*a*) the regenerating liquid could be run straight through all the columns in series and collected as before in the receiver at the base of the terminal column, or (*b*) each column could be regenerated separately. The second of these methods was adopted, 500 ml. of 4N-sulphuric acid being passed in an upward direction through each of the first two and the last two columns at the rate of 10 ml./min.

FIG. 2.



The separation of Ce^{III} *and* Nd^{III} *on the multitube column at two different concentrations.*

Analytical Procedure.—Owing to the possibility of the presence of traces of iron in the percolate fractions, an oxalate separation was carried out as the first stage in the analysis. The procedure then adopted was as follows: (*a*) Cerium and neodymium were precipitated as oxalates, ignited to oxides in a porcelain crucible and weighed (Ce all assumed to be CeO_2). (*b*) The weighed oxides were brought into solution with sulphuric acid and hydrogen peroxide, the hydrogen peroxide boiled off, the cerium oxidised to the quadrivalent state with ammonium persulphate and silver nitrate in sulphuric acid solution, and ceric ion titrated with ferrous ammonium sulphate and potassium permanganate. (*c*) The neodymium content of the fractions was calculated by difference.

Results.—Two full-scale runs were made with total concentrations approximately M and M/4, the ratio of Ce^{III}/Nd^{III} in each case being 8·5 : 1. The run at the higher concentration took 22½ hours, and that at the lower concentration 47½ hours.

In the run with M-solution the leading fractions showed a considerable increase in the proportion of neodymium, the ratio of neodymium to cerium having been more than doubled. As was to be expected, this ratio became less until, towards the end of the run, a sharp increase was noticed. This increase was unexpected, but an estimation of the concentration gradient in the solution leads to a likely explanation. As the apparatus was initially filled with water, there will have been a decrease in the concentration of rare-earth ions at the head of the advancing solution. The final washing with water will have caused a similar decrease in concentration at the tail of the solution, and it may well be this dilution effect which produced a more favourable neodymium/cerium ratio. This conclusion was confirmed by the results of the run at the lower concentration in which a significant increase in the degree of separation was achieved (Fig. 2).

Regeneration of the first and the last two columns showed, in both runs, that the ratio of Nd^{III} ions

to $Ce^{\cdots}$ ions held on the exchanger increased towards the terminal column. Each column, however, contained a greater proportion of $Ce^{\cdots}$ ions than did the original solution.

Discussion.

A partial separation of rare-earth ions has been achieved by preferential ionic exchange on a column. Runs with Ce(III) and Nd mixtures have shown definitely that the former ion, of larger Goldschmidt radius, is relatively more strongly held. This is in agreement with the general experience of Jenny (*loc. cit.*) but contrary to that of Russell and Pearce (*loc. cit.*). No data are available on the relative hydrations of these ions.

The concentration of the solution has a marked influence on the degree of separation achieved, but this effect works in the direction which makes large-scale application difficult because of the large volumes involved. Russell and Pearce (*loc. cit.*) and Bray (*J. Amer. Chem. Soc.*, 1942, **64**, 954) have shown that changes in the ratio of the ions in the liquid phase affect the relative accumulation of ions on the zeolite, but there is no evidence that, with mixtures of ions of the same valency, a change in the absolute concentration without any alteration in the relative ratios of ions in solution would have any such effect. In fact, Ivanov and Gapon (*J. Physical Chem. U.S.S.R.*, 1941, **45**, 659) have concluded that dilution of the solution does not affect the relative uptake in such mixtures, and Renold (*Kolloid-Beih.*, 1935, **43**, 1) has found that, below 0·1N, dilution does not affect the relative uptakes of $Ca^{\cdot\cdot}$ and $K^{\cdot}$ ions. The conclusion in the present work that a reduction in the total concentrations has actually decreased the relative uptake of cerous ion requires explanation, and it is suggested that this lies in the relative dependence of ion hydration on dilution.

It might be suspected that the greater oxidisability of Ce(III) and tendency to formation of ceric ion would bias the results, since it is usually accepted (Walton, *loc. cit.*) that the ion of higher valency is relatively more greatly adsorbed with increased dilution. However, there was no evidence of the presence of ceric ion.

Conclusions.—(1) In a column test with a mixture of cerium and neodymium salts at approximately M-concentration, cerium was more strongly held on Zeokarb H.I.P.; *i.e.*, there was greater retention of the larger ion.

(2) This direction of separation is contrary to the experience of Russell and Pearce (*loc. cit.*) who, using a complex mixture of rare-earth ions, found that ions of smaller Goldschmidt radius were more strongly held.

(3) The degree of separation of these two ions was greater in a test with M/4-solution.

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New Road, Mitcham Junction, Surrey. [*Received, August 15th*, 1947.]

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254. *Derivatives of* 2 : 4 : 6-*Trinitrotoluene : Monoreduction of Polynitro-compounds.*

By G. D. Parkes and A. C. Farthing.

The reduction of 2 : 4 : 6-trinitrotoluene derivatives has been studied, and a method for monoreduction at the 4-position has been worked out. In this and other ways routes to new derivatives of this compound have been made available.

One of the commonest reduction products of 2 : 4 : 6-trinitrotoluene (T.N.T.) is 2 : 6-dinitro-*p*-toluidine (see Tiemann, *Ber.*, 1870, **3**, 218; Beilstein, *Ber.*, 1880, **13**, 243; Holleman and Boeseken, *Rec. Trav. chim.*, 1897, **16**, 425; Brand and Eisenmenger, *J. pr. Chem.*, 1913, **87**, 487; Körner and Contardi, *Gazzetta*, 1917, **47**, i, 228; Brady, Day, and Reynolds, *J.*, 1929, 2264; McGookin, Swift, and Tittenson, *J. Soc. Chem. Ind.*, 1940, **59**, 92). All these methods involve the use of ammonium hydrogen sulphide. The presence of a large amount of alkali has an adverse effect by producing coloured complexes with T.N.T. and by interfering with intermediate reduction products. Further, poly-component systems of molecular compounds may be formed which, along with the impurities normally resulting from organic reactions, make isolation of products difficult. Also, most of the methods given involve heterogeneous mixtures.

The function of the base may be catalytic and, if so, only a trace should be necessary. As

T.N.T. is extremely soluble in dioxan the use of this solvent was tried along with only a trace of ammonia, and it was then found that reduction with comparatively crude hydrogen sulphide took place smoothly with deposition of sulphur and a good yield of 2 : 6-dinitro-*p*-toluidine was obtained.

By this means 2 : 6-dinitrotoluene is made much more readily accessible, and many other derivatives can be obtained by diazotisation; *e.g.*, 4-iodo-2 : 6-dinitrotoluene was prepared in quantitative yield. The method has been found to be adequate for 2 : 4 : 6-trinitrophenyl compounds and it can also be applied to such substances as 2 : 4-dinitrotoluene and 2 : 4-dinitrostilbene. A mixture of 2 : 4- and 2 : 6-dinitrotoluene (Gibson, Duckham, and Fairbairn, *J.*, 1922, **121**, 270) gave 2-nitro-*p*-toluidine, the 2 : 6-isomer being unaffected. This provides another route to 2 : 6-dinitrotoluene.

6-Nitro-2 : 4-diaminotoluene (Tiemann, *loc. cit.*; Ruggli and Zaeslin, *Helv. Chim. Acta*, 1936, **19**, 434; Brady, Day, and Reynolds, *loc. cit.*) has been monoacetylated in good yield by means of acetic anhydride in acetone, diazotisation and deamination giving 2-nitro-*p*-toluidine. A series of nitro-amines may thus be obtained from T.N.T.

Experimental.

2 : 6-*Dinitro*-p-*toluidine*.—T.N.T. (100 g.) was dissolved in dioxan (250 c.c.) and three drops of ammonia (*d* 0·88) were added with shaking. Hydrogen sulphide (from dilute hydrochloric acid and ferrous sulphide, washed with aqueous sodium hydrogen sulphide and dried over calcium chloride), was passed in at a moderate rate. Reaction set in after 3—5 mins. (with gentle warming, if necessary), the temperature being kept below 40°. Monoclinic sulphur was precipitated and the hydrogen sulphide stream was continued until the temperature fell. A further three drops of ammonia were added, and the procedure repeated. Sulphur (39 g.) was filtered off and washed with dioxan and ether; 3 drops of concentrated hydrochloric acid were added to filtrate and washings, and the solvent was removed by distillation under reduced pressure from a water-bath. The residual solid was powdered and dried (90 g., 100%). It contained a trace of T.N.T. as shown by Janovsky's test. This was used for diazotisation without purification. Isolation could also be achieved as hydrochloride by passing dry hydrogen chloride into the dioxan solution, 50—60% of almost pure hydrochloride being obtained; m. p. 172° (acetyl derivative, m. p. 221·5°) (Found: C, 45·2; H, 3·65. Calc. for $C_9H_9O_5N_3$: C, 45·2; H, 3·76%).

4-*Chloro- and* 4-*Iodo*-2 : 6-*dinitrotoluene*.—2 : 6-Dinitro-*p*-toluidine hydrochloride (47 g.; 0·2 mol.) was finely powdered and suspended in concentrated hydrochloric acid (500 c.c.). A saturated aqueous solution of sodium nitrite (16 g.) was added at 0°. After one hour a cloudy solution was formed. Three-fourths of this solution was added with stirring to a cold solution of cuprous chloride (10 g.) in concentrated hydrochloric acid (100 c.c.) and one-fourth to a cold solution of potassium iodide (20 g.) in water (100 c.c.). The respective halides floated to the top of the solution and were filtered off, washed, and dried; yields were 15 g. (0·05 mol.) of 4-iodo-2 : 6-dinitrotoluene and 33 g. (0·15 mol.) of chloro-compound. The latter, crystallised from alcohol, had m. p. 76° (Cohen and McCandlish, *J.*, 1905, **87**, 1265).

6-*Nitro*-2 : 4-*diaminotoluene*.—Ruggli and Zaeslin's method (*loc. cit.*) was found satisfactory: yield, 78%, m. p. 129° after distillation in a vacuum and repeated recrystallisation from water (Ruggli and Zaeslin give 135°).

2-*Nitro*-6-*acetamido*-p-*toluidine*.—(*a*) 6-Nitro-2 : 4-diaminotoluene (5 g.) was finely ground and cooled in ice–salt. Acetic anhydride (3·05 g., 1 mol.) was added dropwise with stirring. A viscous mass was formed which then solidified. It was finely ground and extracted once with toluene (charcoal). From the toluene the *acetyl* compound was obtained as an orange solid, m. p. 155°, after several recrystallisations (Found: C, 51·7; H, 5·25. $C_9H_{11}O_3N_3$ requires C, 51·7; H, 5·20%). The portion soluble in toluene was extracted with alcohol to yield white fluffy needles of the *diacetamido*-compound, m. p. 265° (Found: C, 50·6; H, 5·2. $C_{11}H_{13}O_3N_3$ requires C, 50·6; H, 5·4%). A small amount of starting material was obtained from the mother-liquor.

(*b*) 6-Nitro-2 : 4-diaminotoluene (5 g.) was vigorously shaken with 2N-sodium hydroxide (20 c.c.) and ice (20 g.). Acetic anhydride (3·05 g.) was added slowly with shaking. The product was repeatedly recrystallised from toluene and from 80% alcohol to give poor yields of the monoacetyl derivative.

(*c*) 6-Nitro-2 : 4-diaminotoluene (5 g.) was dissolved in dried and distilled acetone. Acetic anhydride (3·05 g.) in similar acetone (10 c.c.) was slowly added with stirring. After 30 mins. at room temperature the solvent was removed on the water-bath, yielding the monoacetyl compound as a solid (5·3 g.). This was pure after one crystallisation from 80% alcohol or toluene (4·9 g., 92%).

(*d*) 6-Nitro-2 : 4-diaminotoluene (5 g.) was heated on a water-bath in 25 c.c. of acetic acid for 60 mins. The acetic acid was removed at room temperature over sodium hydroxide. An intractable gum was obtained.

6-*Nitro*-o-*toluidine*.—2-Nitro-6-acetamido-*p*-toluidine (2 g.) was shaken with concentrated hydrochloric acid (15 c.c.) and ethyl acetate (1 c.c.). The suspension was cooled to 0° and the theoretical amount of saturated aqueous sodium nitrite added gradually with stirring. After 30 mins. the solution was slowly poured into alcohol (20 c.c.) boiling on a water-bath. When evolution of nitrogen and acetaldehyde ceased the solution was evaporated in a vacuum over sodium hydroxide. The tar produced was extracted with hot 2N-hydrochloric acid (charcoal) and precipitated with ammonia. Yellow-orange 6-nitro-*o*-toluidine was filtered off (0·8 g., 54%) and crystallised from 30% acetic acid; m. p. 90°. It was identical with an authentic specimen (Bernthsen, *Ber.*, 1882, **15**, 3017; Green and Lawson, *J.*, 1891, **59**, 1013). Its acetyl derivative had m. p. 156° after crystallisation from aqueous alcohol.

2 : 6-*Dinitro*-4-*aminostilbene.*—2 : 4 : 6-Trinitrostilbene (7 g.) (Bishop and Brady, *loc. cit.*) in dioxan (50 c.c.) plus one drop of aqueous ammonia (*d* 0·880) was treated with hydrogen sulphide as in the reduction of 2 : 6-dinitro-*p*-toluidine. Sulphur (1·8 g.) was filtered off. Removal of solvent at 50° in a vacuum gave a hard red gum which resisted purification. The product was isolated as hydrochloride by precipitation with dry hydrogen chloride. The yellow precipitate was filtered off after standing overnight and washed with dioxan and dry ether; m. p. 173° (decomp.) (2·6 g.). More hydrochloride was obtained by leaving the filtrate in a vacuum over concentrated sulphuric acid for 36 hours. The hydrochloride was shaken with ether–dilute aqueous ammonia until it had dissolved. The ethereal layer was dried, and solvent removed to give orange needles (4·5 g., 70%) of the 4-*amino*-compound, which were recrystallised from 80% alcohol; m. p. 157° (Found : C, 58·7; H, 4·05. $C_{14}H_{11}O_4N_3$ requires C, 58·9; H, 3·86%). The *acetyl* derivative formed pale yellow needles, m. p. 208·5°, from 80% alcohol (Found : C, 58·5; H, 4·1. $C_{16}H_{13}O_5N_3$ requires C, 58·6; H, 4·0%).

2 : 6-*Dinitrostilbene.*—2 : 6-Dinitro-4-aminostilbene (1 g.) was dissolved in hot acetic acid (10 c.c.) and added to concentrated sulphuric acid (10 c.c.) cooled in ice–salt. Dry, finely powdered sodium nitrite (0·3 g.) was added slowly at 0° with stirring. After two hours the deep orange solution was slowly added to alcohol (60 c.c.) boiling on a water-bath. When no more acetaldehyde came off the mixture was added to 400 c.c. of cold water. After standing overnight, the precipitate was filtered off and taken up in hot alcohol (5 c.c.), charcoal was added, and then water slowly. A tar came down, followed by yellow-orange needles. More charcoal was added, and the solution boiled and filtered. The yellow-orange needles obtained, recrystallised from aqueous alcohol (charcoal), had m. p. 111° (0·1 g., 12%) (Found : C, 62·0; H, 3·9; N, 10·7. Calc. for $C_{14}H_{10}O_4N_2$: C, 62·2; H, 3·7; N, 10·4%). Janovsky's test gave a delicate green colour as for 2 : 6-dinitrostilbene (Pfeiffer, *Ber.*, 1906, **39**, 1304; *Annalen*, 1916, **411**, 72).

Reduction of 2 : 4 : 6 : 3′-Tetranitrostilbene (5 g.) (Brady and Bishop, *loc. cit.*) by the method used for 2 : 4 : 6-trinitrostilbene gave sulphur (1 g. Calc. : 1·3 g.). Attempts to work up the mixture failed and no crystalline product was obtained.

Reduction of 2 : 4 : 6-*Trinitrobenzoic Acid* (*Org. Synth.*, **2**, 95).—2 : 4 : 6-Trinitrobenzoic acid (2 g.) was powdered, suspended in water (20 c.c.), and ammonia (*d* 0·880) added slowly with stirring until a permanent red colour formed and all acid dissolved. Hydrogen sulphide was passed in for one hour and the solution left overnight. Very finely divided sulphur was filtered off, and the filtrate shaken with ether. Concentrated hydrochloric acid was slowly added until the ethereal layer was a much deeper orange than the aqueous layer. The former layer gave an orange solid (1·4 g., m. p. 176—180°), and the latter gave ammonium chloride and a trace of red solid. The product was extremely soluble in water, alcohol, acetone, ethyl acetate, or dioxan, very slightly soluble in hot benzene, toluene, or chloroform, and insoluble in light petroleum (b. p. 80—100°). It could not be distilled or sublimed. After six precipitations from hot benzene (95%) and alcohol (5%) by light petroleum (b. p. 80—100°), a constant m. p. of 218° (decomp.) was obtained (Found : C, 35·5; H, 2·3. Calc. for $C_7H_3O_6N_3$: C, 37·0; H, 2·2%). An acetyl derivative of similar solubility properties was made; white plates, m. p. 220° (decomp.). On diazotisation and reaction with hot alcohol a white acidic solid, m. p. 203°, was obtained.

Reduction of 2-(2′ : 4′ : 6′-*Trinitrophenyl*)*ethanol by Hydrogen Sulphide.*—The ethanol (Vender, *Gazzetta*, 1915, **45**, ii, 97) (5 g.) in dioxan (25 c.c.) and one drop of aqueous ammonia (*d* 0·880) was reduced with hydrogen sulphide as was T.N.T. Sulphur (1·7 g.) was filtered off, and a white solid, m. p. 161—165° (3·7 g.), precipitated by dry hydrogen chloride. The white solid was treated with ether–dilute ammonia, and a yellow solid (3·2 g.) isolated from the ether. It was recrystallised from acetic acid and then from toluene, forming yellow fluffy needles of 2-(2′ : 6′-*dinitro*-4′-*aminophenyl*)*ethanol* (Found : C, 42·6; H, 4·1. $C_8H_9O_5N_3$ requires C, 42·5; H, 4·0%). The *diacetyl* derivative formed white plates, m. p. 139°, from aqueous alcohol (Found : C, 46·2; H, 4·3. $C_{12}H_{13}O_7N_3$ requires C, 46·3; H, 4·2%).

Reduction of 2-(2′ : 4′ : 6′-*Trinitrophenyl*)*ethyl Acetate by Hydrogen Sulphide.*—The acetate (2 g.) (Vender, *loc. cit.*) in dioxan (10 c.c.) and one drop of aqueous ammonia (*d* 0·880) was reduced with hydrogen sulphide as was T.N.T. The product was isolated as described for trinitrophenylethanol to give an orange solid (1·7 g., 94%), which crystallised from aqueous alcohol and then benzene as yellow needles, m. p. 116·5° (Found : C, 44·5; H, 4·0. $C_{10}H_{11}O_6N_3$ requires C, 44·4; H, 4·1%). Attempts to acetylate this 4′-*amino*-compound gave intractable gums.

2 : 4 : 6 : 2′ : 4′ : 6′-*Hexanitrodibenzyl.*—T.N.T. (5 g.) and sodium carbonate (5 g.) in water (150 c.c.) were heated on the water-bath and air was passed through the mixture for 5 hours. It was then cooled and centrifuged, and the solid (6 g.) was dried in a vacuum over concentrated sulphuric acid and extracted with benzene (Soxhlet). The residue, 2·4 g. of finely divided black solid, could not be purified and exploded on heating. The benzene extract was evaporated to 40 c.c. and then left in a vacuum over concentrated sulphuric acid. White needles were formed at the bottom of the solution and then T.N.T. on the walls of the dish. The white needles were separated (0·2 g., 4%) and recrystallised from toluene; m. p. 216—217° (decomp.). The residual benzene gave only T.N.T. on total evaporation.

Reduction of 2-(2′ : 4′ : 6′-*Trinitrostyryl*)*furan by Hydrogen Sulphide.*—The furan (Pastak, *Bull. Soc. chim.*, 1926, **39**, 72, 77) (5 g.) in dioxan (20 c.c.) was treated with hydrogen sulphide in the presence of a trace of ammonia; only slight reaction took place after 6 hours, yielding a trace of sulphur (0·1 g.). The experiment was repeated with a drop of 2N-sodium hydroxide instead of ammonia. No reaction took place, so a drop of aqueous ammonia (*d* 0·880) was added. The temperature immediately rose and was kept below 40° with cooling. When the temperature fell, sulphur was filtered off (1·5 g.). The filtrate was dissolved in ether (200 c.c.) and repeatedly shaken with water. The ether was removed, leaving a red gum, from which, by recrystallisation from aqueous alcohol, very unstable red needles were isolated, m. p. 133°, not identical with the starting material. The m. p. was found to have fallen substantially after the substance had been kept for an hour in a vacuum. Immediately after recrystallisation the red needles gave an olive-green colour with concentrated sulphuric acid. The substance could not be acetylated.

Colour reactions with alkali of derivatives of T.N.T. (Janovsky's test).

Colours observed when 10—20 mg. of the compound were dissolved in about 5 c.c. of solvent and one drop of alkali added. The colour was formed more quickly and was more intense with sodium hydroxide instead of aqueous ammonia.

Compound.	EtOH and NaOH.		$COMe_2$ and NaOH.	
	Cold.	Hot.	Cold.	Hot.
2 : 4-Dinitrotoluene	Blue	Deep red	Blue	Deep red
2 : 4-Dinitrostilbene	Green	Red-brown	Deep blue	Purple
T.N.T.	Red	Red	Red	Red
4-Chloro-2 : 6-dinitrotoluene	Red; blue fluorescence	Deep red	Pink	Magenta
4-Iodo-2 : 6-dinitrotoluene	Blue-violet	Black	—	—
2 : 4 : 6-Trinitrostilbene	Bright crimson	Deep red	Bright crimson	Deep red
2 : 4 : 6 : 3′-Tetranitrostilbene	Bright red	Deep red	Bright red	Deep red
Dinitroaminostilbene	Orange red	Deep red-brown	—	—
Acetyl derivative	Yellow	Yellow	Yellow	Orange-yellow
2 : 6-Dinitro-4-acetamidotoluene	Yellow	Yellow	Yellow	Orange-yellow
Dinitroaminophenylethanol	Green	Grey-green	Olive-green	Dark olive-green
Diacetyl derivative	Green	Green-yellow	Yellow	Yellow
Trinitrophenylethanol	Bright red	Deep red	Bright red	Deep red
Trinitrophenylethyl acetate	Bright red	Deep red	Bright red	Deep red
Dinitroaminophenylethyl acetate	Red	Deep red	—	—
Ammonium or sodium trinitrobenzoate	Red	Deep red	Red	Deep red
2 : 4 : 6-Trinitrobenzaldehyde	Red	Deep red	Red	Deep red
Anil of above	Red	Deep red	Red	Deep red
2-Furylidene-T.N.T.	Red	Deep red	Red	Deep red

The authors wish to thank Professor Sir Robert Robinson, P.R.S., for his interest and valuable advice.

Dyson Perrins Laboratory, Oxford. [*Received, September 26th*, 1947.]

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255. *The Galactomannan of Carob-seed Gum (Gum Gatto).*

By E. L. Hirst and J. K. N. Jones.

Gum gatto (alternative names : locust-bean gum, gum tragon, St. John's bread, carob-seed gum) has been shown to be a branched chain polysaccharide built up of D-mannose residues (*ca.* 84%) and D-galactopyranose residues (*ca.* 16%). The methylated gum yields on methanolysis 2 : 3 : 4 : 6-tetramethyl methyl-D-galactoside (*ca.* 1 part), 2 : 3 : 6-trimethyl methyl-D-mannoside (*ca.* 4 parts), and 2 : 3-dimethyl methyl-D-mannoside (*ca.* 1 part) identified as the crystalline furanolactone of 2 : 3-dimethyl D-mannonic acid which yields a crystalline *phenylhydrazide*. The dimethyl D-mannonic acid on oxidation with periodic acid yields formaldehyde, its constitution being thereby proved.

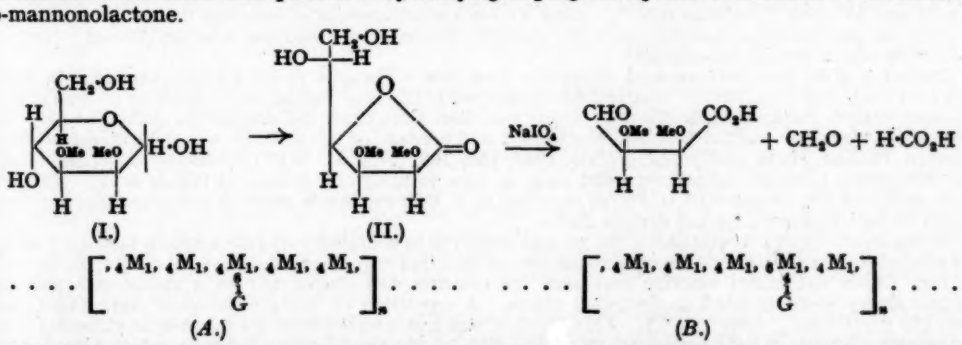
Galactomannans are of common occurrence in Nature. They have been isolated from various seeds such as the fenugreek (*Trigonella foenum-græcum*) (Daoud, *Biochem. J.*, 1932, **26**, 255), the lucerne seed (Hirst, Jones, and Walder, *J.*, 1947, **1443**), the coffee bean (Wise and Appling, *Ind. Eng. Chem. Anal.*, 1944, **16**, 28), and the carob seed (Spada, *Atti Soc. nat. mat. Modena*, 1939, **70**, 20; Lew and Gortner, *Arch. Biochem.*, 1943, **1**, 32). The polysaccharides can be isolated from these sources by extraction with cold dilute sodium hydroxide from which they may be precipitated as a copper complex by addition of Fehling's solution. In this and other respects these galactomannans resemble mannan A of the ivory nut (Klages, *Annalen*, 1934, **509**, 159; 1935, **512**, 185). The galactomannans are utilised in the paper industry since they form thick mucilaginous dispersions which, when added to the cellulose suspension, increase the strength of the resultant paper. A mucilage frequently used for this purpose is the one obtained from carob-seed gum (gum gatto) which forms the subject of the present inquiry. The commercially obtainable material is a white powder which does not dissolve in water, but swells to a thick mucilaginous mass which is soluble in dilute sodium hydroxide with loss of viscosity. The alkaline solution can then be filtered and the purified gum isolated either as the insoluble copper complex by addition of Fehling's solution, or as the pure polysaccharide by precipitation in acidified alcohol. The gum is prepared from the seeds of the bean from *Ceratonia siliqua*, commonly found growing in Crete, Greece, Portugal, the Balearic Islands, and Cyprus.

In the course of the present work it was not found possible to hydrolyse the polysaccharide without some decomposition of the resulting sugars, since it dissolved only slowly in the acid used for hydrolysis. A small quantity of the hydrolysis mixture was examined on the paper

chromatogram by the method of Partridge (*Nature*, 1946, **158**, 270). Galactose and mannose were detected, the latter predominating. A quantitative determination of these sugars by the method described of Hirst, Jones, and Woods (*J.*, 1947, 1048) showed the presence of some 86% of D-mannose and 14% of D-galactose. With a view to determining whether the polysaccharide was of the branched chain type or not a sample of the purified material was oxidised with potassium periodate. The yield of formic acid indicated that hydroxyl groups were present on each of three adjacent carbon atoms in approximately one sugar residue in six, and therefore one sugar residue in six was an end group or was linked through carbon atoms one and six only. The polysaccharide which had been oxidised with potassium periodate was submitted to hydrolysis and the products examined on the paper chromatogram. No sugar other than a mere trace of mannose was detected, and it follows that neither galactopyranose nor mannopyranose residues combined through C_2 or through any two of the carbon atoms C_2, C_3, and C_4 were present (see Halsall, Hirst, and Jones, *J.*, 1947, 1427). Sugar residues linked in any of these ways contain no α-glycol groupings and if present they would have escaped oxidation by the periodate reagent.

The acidic hydrolysis of the polysaccharide was unsatisfactory and attempts were therefore made to hydrolyse the material with enzymes. Some reaction occurred with taka-diastase, but a much more active preparation was the commercial mixture of enzymes known as " Pectinol 10M ", which is used to free fruit juices from pectic acid. This enzyme was found to act on a suspension of gum gatto and to convert it in part into D-mannose, but no D-galactose could be detected. Hydrolysis was slow and incomplete, possibly because the optimum conditions of temperature and pH had not been employed. This enzyme was also capable of degrading the ivory nut mannan A of Klages, but under the experimental conditions employed hydrolysis was again incomplete.

More detailed information about the structure of the polysaccharide was obtained from an examination of its methylated derivative. On hydrolysis the following three sugar derivatives were isolated. (*a*) 2 : 3 : 4 : 6-Tetramethyl D-galactose (*ca.* 1 mol.), identified as its crystalline anilide. (*b*) 2 : 3 : 6-Trimethyl D-mannose (*ca.* 4 mols.) identified as its crystalline anilide. The sugar on oxidation gave crystalline 2 : 3 : 6-trimethyl D-mannonolactone (Haworth, Hirst, and Streight, *J.*, 1931, 1349), further characterised as the corresponding crystalline amide and *phenylhydrazide*. (*c*) A dimethyl hexose (*ca.* 1 mol.) which was mainly, if not entirely, 2 : 3-dimethyl D-mannose (I) since it gave on oxidation a crystalline lactone (II), identical with the product of Goodyear and Haworth (*J.*, 1927, 3136), and yielded when boiled with alcoholic phenylhydrazine a crystalline *phenylhydrazide*. The lactone of Goodyear and Haworth (*loc. cit.*) must be either 2 : 3- or 5 : 6-dimethyl D-mannonolactone from its mode of preparation, and since it differs from the 5 : 6-dimethyl D-mannonolactone which was described by Irvine and Patterson (*J.*, 1914, 898; cf. Wiggins, *J.*, 1946, 13), the lactone of Goodyear and Haworth is almost certainly the 2 : 3-derivative. The constants of (II) were identical with this product, and its identity was placed beyond doubt by a study of its reaction with sodium periodate. This reagent, diagnostic for α-glycols, splits the acid derived from (II) with production of formaldehyde, so proving the presence of hydroxyl groups on C_6 and C_5. Since (II) is a furanolactone it must also possess a hydroxyl grouping on C_4 and is therefore 2 : 3-dimethyl D-mannonolactone.



M and G represent D-mannopyranose and D-galactopyranose residues linked through the numbered carbon atoms.

The isolation of 2 : 3 : 4 : 6-tetramethyl D-galactose, 2 : 3 : 6-trimethyl D-mannose, and 2 : 3-dimethyl D-mannose in the approximate proportions 1 : 4 : 1 proves that all the galactose is

present in the pyranose form as an end group and that it is attached to a D-mannose residue through C_4 or C_6 (contrast Lew and Gortner, *loc. cit.*, who suggest a 1 : 2-linkage). The polysaccharide is highly resistant to acidic hydrolysis, and the mannose residues are therefore probably of the pyranose type. Two main possibilities for the formula of gum gatto are possible. The mannose residues may be in the form of a chain of 1 : 4-linked mannopyranose residues (probably of the β-configuration) with every fifth residue substituted at C_6 by a D-galactopyranose residue (*A*) or, groups of four 1 : 4-β-linked mannopyranose residues may be linked to one another by a D-mannopyranose residue through C_1 and C_6 and a D-galactopyranose residue is linked to C_4 of this sugar residue (*B*). The main chain structure of (*A*) is identical with the structure of ivory nut mannan which was postulated by Klages (*loc. cit.*).

It may be noted that other workers have found different ratios of galactose to mannose in carob-seed gum, and figures of 27 : 73 (Spada, *loc. cit.*), 20 : 80 (Wise and Appling, *loc. cit.*), and 18 : 82 (Lew and Gortner, *loc. cit.*) have been given. These differences suggest that the galactose/mannose ratio may vary with the origin of the gum and that the glactose is stored by attaching it randomwise to the main chain of mannose residues. The possibility remains, however, that the gum is a mixture of polysaccharides, such as a galactomannan and a mannan, and that the variations in galactose content are due to variations in the amount of the galactomannan component. Fractionation of the methyl ether derivative, however, failed to achieve any obvious separation into components possessing different physical properties, a result which was verified from an examination of the products of hydrolysis of the various fractions of the methyl ether derivative (see experimental section).

Experimental.

(All boiling points recorded in distillation are bath temperatures.)

The sample of gum gatto used in this work was supplied by Messrs. Ellis Jones & Co. Ltd., Stockport. It was a white powder which contained traces of brown material, and had been obtained from the seeds of the bean from *Ceratonia siliqua*. The sample used in these experiments was of Cyprian origin and was free from husks. The crude gum when boiled with 12% hydrochloric acid gave 1·9% of furfuraldehyde, equivalent to the presence of some 3·8% of pentosan (Found : N, 1·1; OMe, 0·3; ash, 1·0%).

The polysaccharide swelled in cold water but did not dissolve. It was purified by dissolving it in dilute aqueous sodium hydroxide, the gum being precipitated by pouring the filtered solution into alcohol acidified with hydrochloric acid. The product, a white powder, was washed with alcohol until it was neutral, then with ether, and dried under diminished pressure. This material was free from ash and nitrogen and gave a negligible yield of furfuraldehyde when boiled with 12% hydrochloric acid. It gave an insoluble copper complex on addition of Fehling's solution to its solution in aqueous sodium hydroxide (cf. ivory nut mannan, *loc. cit.*).

Hydrolysis of Purified Gum Gatto.—The purified gum (3·2 g.) was heated with N-sulphuric acid (50 c.c.) at 93°; it gradually dissolved, leaving a small amount of flocculent impurity. It was not possible to follow completely the change of optical rotation, owing to darkening of the solution. The solution was filtered, and the filtrate neutralised with barium carbonate and again filtered; $[\alpha]_D^{20°}$ + 28° (*c*, 1·1 in water). A sample of the solution was examined on a paper chromatogram (Partridge, *loc. cit.*), and no sugars other than galactose and mannose were detected. Iodimetric titration of the solution showed the presence of some 3·4 g. of reducing sugar, calculated as hexose. A solution containing 80% of D-mannose and 20% of D-galactose would have $[\alpha]_D^{20°}$ + 28° (Found : D-mannose 86%, determined as the phenylhydrazone, and D-galactose 14%, determined as the phenylmethylhydrazone).

The polysaccharide (1 g.) underwent some hydrolysis with loss in viscosity of the solution on standing at pH5 and 35° with " Pectinol 10M ". After 5 days a small amount of insoluble material was removed by filtration and mannose determined as the phenylhydrazone in the residual solution (Found : 0·45 g.; hydrolysis was therefore incomplete).

Oxidation of the Purified Gum with Potassium Periodate.—The gum (1·002 g.) was suspended in water (100 c.c.) contained in a 500 c.c. steamed-out stoppered bottle, and potassium chloride (5 g.) was added. Aqueous sodium metaperiodate (35 c.c.; 0·3M) was then added and the suspension shaken at 20°. At intervals samples were withdrawn and the formic acid content of the solution was determined (Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785) [Found : 1010 (170 hours), 970 (193 hours), 965 (220 hours, constant value), recorded as g. of gum yielding one g.-mol. of formic acid]. The last figure indicates the presence of 16·8% of terminal or 1 : 6-linked sugar residues which contain hydroxyl groups on each of three adjacent carbon atoms.

Methylation.—The polysaccharide (30 g.) was dissolved in sodium hydroxide solution (400 c.c.; 40%) and methylated in an atmosphere of nitrogen by the addition of methyl sulphate (360 c.c.) with vigorous stirring. After the initial reaction was over the solution was evaporated on a steam-bath and the residual slurry re-methylated as described above. A repetition of this process gave methylated gum gatto (30 g.) (Found : OMe; 43%). This product was fractionated from its solution in chloroform by portionwise addition of light petroleum (b. p. 40—60°), giving the following fractions which have almost identical properties : fraction (A) (10 g.), $[\alpha]_D^{20°}$ − 11° (*c*, 0·85 in water) (Found : OMe, 43%); Fraction (B) (12 g.), $[\alpha]_D^{21°}$ − 12° (*c*, 1·1 in water) (Found : OMe, 44·1%); Fraction (C) (7 g.), $[\alpha]_D^{19°}$ − 11° (*c*, 0·1 in water) (Found : OMe, 44·0%). The methylated material was consequently held to be essentially homogeneous or an inseparable mixture. Each fraction was therefore hydrolysed separately and the products of hydrolysis examined in order to detect any differences in the three fractions.

Examination of Fraction (A).—This fraction (3·10 g.) was boiled with methanolic hydrogen chloride (100 c.c.; 2%) for 10 hours until the rotation became constant; $[\alpha]_D^{20°}$ + 60·7°. The solution was neutralised with aqueous sodium hydroxide (30%) and concentrated to a syrup, which was dissolved in water (30 c.c.) and extracted exhaustively first with light petroleum (b. p. 40—60°) and then with ether. The aqueous portion was then evaporated to dryness and the residue was exhaustively extracted with chloroform.

The syrup (1·62 g., $n_D^{16°}$ 1·4575) obtained on evaporation of the light petroleum extract was distilled in a vacuum yielding: fraction (1) (1·06 g.), b. p. 130°/0·1 mm., $n_D^{17°}$ 1·4560 (Found: OMe, 57·7%). The ethereal extract (1·55 g.) was added to the still residue and the distillation was continued yielding: fraction (2) (1·36 g., b. p. 160°/0·1 mm., $n_D^{18°}$ 1·4655 (Found: OMe, 52·6%). The chloroform extract (0·2 g.) was then added to the still and the distillation continued yielding: fraction (3) (0·45 g.), b. p. 160/0·1 mm., $n_D^{17°}$ 1·4715 (Found: OMe, 45·5%). The final still residue weighed 0·4 g. (Found: OMe, 42%).

Fraction (1) (1·05 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{18°}$ + 89° (initial value) ⟶ + 59° (constant value). This figure taken in conjunction with the methoxyl content corresponds to the presence of some 59% of 2 : 3 : 4 : 6-tetramethyl D-galactose ($[\alpha]_D$ + 118°) in fraction (1). The solution was neutralised with aqueous sodium hydroxide (30%) and extracted exhaustively with chloroform. The sugars (0·9 g.) obtained on evaporation of the solvent were converted into their anilides by boiling them with ethyl alcohol containing just under 1 mol. of aniline, the slight deficiency in aniline being found to result in a more stable and less discoloured anilide. Concentration of the alcohol gave 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (total yield, 0·67 g.), m. p. 193°, not depressed on admixture with an authentic specimen. This yield of anilide corresponds to the presence of 0·55 g. of tetramethyl D-galactose in fraction (1). The mother liquor from the preparation of the anilide gave in small yields 2 : 3 : 6-trimethyl D-mannose anilide, m. p. 134°, not depressed on admixture with an authentic specimen (Haworth, Hirst, and Streight, *loc. cit.*).

Fraction (2) (1·35 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{18°}$ + 15·7° ⟶ − 9° (3 hours, constant value). The solution was neutralised with aqueous sodium hydroxide (30%) and extracted exhaustively with chloroform. The syrup {1·16 g., $n_D^{18°}$ 1·4740, $[\alpha]_D^{20°}$ − 10° (*c*, 1·1 in water)} obtained on removal of the chloroform gave, when heated with alcoholic aniline, 2 : 3 : 6-trimethyl D-mannose anilide, in high yield, m. p. 133°, not altered on recrystallisation from benzene–light petroleum (b. p. 40—60°). The sugar (0·4 g.) on oxidation with bromine water was converted into the lactone of 2 : 3 : 6-trimethyl D-mannonic acid (0·4 g., $n_D^{16°}$ 1·4710) which was isolated in the usual manner and purified by distillation; b. p. 180°/0·5 mm. The lactone crystallised in the receiver and had m. p. 79—80° after recrystallisation from ether; $[\alpha]_D^{20°}$ + 73° ⟶ + 67° (200 hours, constant value) (Found: C, 48·9; H, 7·3; OMe, 41·2. Calc. for $C_9H_{16}O_6$: C, 49·1; H, 7·3; OMe, 42·2%). The lactone gave with liquid ammonia a crystalline amide, m. p. 130°, which was not suitable for characterisation, owing to the difficulty encountered in recrystallising it. The *phenylhydrazide* (0·15 g.), prepared by heating the lactone (0·1 g.) with an alcoholic solution of phenylhydrazine (0·1 g.), was more satisfactory as a derivative; m. p. 132° after recrystallisation from alcohol (Found: C, 54·9; H, 6·8; N, 8·2; OMe, 27·4. $C_{15}H_{24}O_6N_2$ requires C, 54·9; H, 7·3; N, 8·5; OMe, 28·4%) (cf. Haworth, Hirst, and Streight, *loc. cit.*).

Fraction (3) and the still residue were combined and the whole (0·8 g.) hydrolysed with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 70° (initial value) ⟶ − 15° (3 hours; constant value). The solution was neutralised with aqueous sodium hydroxide and evaporated to dryness, and the residue extracted exhaustively with chloroform. The syrupy mixture of sugars (0·7 g.) (Found: OMe, 34%) obtained on evaporation of the chloroform was oxidised with bromine water. The mixed lactones (0·5 g.), when heated with alcoholic phenylhydrazine, gave the phenylhydrazide of 2 : 3 : 6-trimethyl D-mannonic acid, m. p. and mixed m. p. 132°, and the *phenylhydrazide* of dimethyl D-mannonic acid, m. p. 170°, which were separated by recrystallisation from alcohol (Found: for the substance of m. p. 170°: C, 53·3; H, 7·6; N, 8·8; OMe, 19·1. $C_{14}H_{22}O_6N_2$ requires C, 53·2; H, 7·0; N, 8·9; OMe, 19·7%).

Hydrolysis of Fraction B.—The methylated gum (5·75 g.) was dissolved in methanolic hydrogen chloride (100 c.c. 3%) and the solution boiled under reflux for 17 hours; $[\alpha]_D^{18°}$ + 14° (initial value), + 56° (2¼ hours), + 60° (17 hours, constant value). The solution was neutralised with N-sodium hydroxide, and the methyl alcohol was removed by distillation. The aqueous solution so obtained was extracted exhaustively with chloroform which removed 5·89 g. of material; it was then evaporated to dryness, and the residue extracted with acetone, evaporation of which yielded a further quantity of syrupy glycosides (0·13 g.).

The material in the chloroform extract was distilled in a vacuum yielding: fraction (4) (1·04 g.), b. p. 125°/0·1 mm., $n_D^{20°}$ 1·4540 (Found: OMe, 59·9%); fraction (5) (0·36 g.), b. p. 150°/0·01 mm., $n_D^{20°}$ 1·4602 (Found: OMe, 54·0%); fraction (6) (1·75 g.), b. p. 150°/0·1 mm., $n_D^{20°}$ 1·4629 (Found: OMe, 51·7%); fraction (7) (1·12 g.), b. p. 150—200°/0·01 mm., $n_D^{17°}$ 1·4740 (Found: OMe, 43·0%); still residue (0·12 g.).

Examination of the Fractions.—Fraction (4) (1·63 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 105° (initial value) ⟶ + 77° (constant value). This equilibrium value corresponds to the presence in this fraction of some 68% of 2 : 3 : 4 : 6-tetramethyl D-galactose ($[\alpha]_D$ + 118°) and 32% of 2 : 3 : 6-trimethyl D-mannose. The solution was neutralised with aqueous sodium hydroxide (30%) and then extracted exhaustively with ether. Removal of the ether left slightly impure 2 : 3 : 4 : 6-tetramethyl D-galactose (1·41 g.), identified as the anilide, m. p. 195°, not depressed on admixture with an authentic specimen.

Fraction (5) (0·35 g.) on hydrolysis for 2 hours with N-sulphuric acid (10 c.c.) gave a mixture of sugars having $[\alpha]_D^{20°}$ + 7°. This on conversion into the corresponding anilides gave a very small quantity of 2 : 3 : 4 : 6-tetramethyl D-galactose anilide (30 mg.), m. p. 193°, and a larger quantity of 2 : 3 : 6-trimethyl D-mannose anilide (120 mg.), m. p. and mixed m. p. 133°, separation being effected by recrystallisation from ether–alcohol.

Fraction (6) (1·7 g.) was hydrolysed with boiling N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 28° ⟶ − 7° (3 hours, constant value). The solution was neutralised with 30% sodium hydroxide and exhaustively

extracted with chloroform. The material extracted weighed 2·4 g., and a portion of it (0·6 g.), when boiled with alcoholic aniline, gave in good yield (0·6 g.) 2 : 3 : 6-trimethyl D-mannose anilide, m. p. and mixed m. p. 132°. A second portion (0·7 g.) was oxidised with bromine water, and the lactone (0·7 g.) was isolated in the usual manner. The product crystallised. It was purified first by distillation at 140°/0·1 mm. and then by recrystallisation from a large volume of ether; m. p. 80°, not depressed on admixture with an authentic example of 2 : 3 : 6-trimethyl D-mannonolactone.

The optical rotation of this sugar fraction taken in conjunction with its methoxyl content and the yields of anilide and lactone indicate that 2 : 3 : 6-trimethyl D-mannose was the sole constituent.

A portion of fraction (7) (0·98 g.) was hydrolysed by boiling it with N-sulphuric acid (10 c.c.); $[\alpha]_D^{20°}$ + 15° (initial value) ⟶ − 14° (4 hours, constant value). The solution was neutralised with 30% sodium hydroxide, 1 drop of acetic acid added to avoid the development of alkalinity, and the solution evaporated to dryness under reduced pressure. The product (0·71 g.) was extracted from the residue by boiling it with acetone. It was a pale yellow syrup which did not crystallise (Found : OMe, 26·3%). No crystalline anilide or phenylhydrazone could be prepared from it. Accordingly, the sugar (0·5 g.) was oxidised with bromine water and the lactone (which was a syrup) was isolated in the usual manner and converted into the phenylhydrazide by boiling it with an alcoholic solution of phenylhydrazine. The product after recrystallisation from alcohol had m. p. 170°, and was identified as the phenylhydrazide of 2 : 3-dimethyl D-mannonic acid (see below).

Hydrolysis of Fraction C.—This fraction (2·56 g.) was boiled with methanolic hydrogen chloride (50 c.c.; 2%) for 15 hours; the rotation was then constant at $[\alpha]_D^{20°}$ + 60°. The solution (cooled in acetone–solid carbon dioxide) was neutralised with ice-cold diazomethane and evaporated under reduced pressure to a syrup (2·76 g., $n_D^{20°}$ 1·4590). This syrup was separated into fractions by continuous extraction in a special apparatus consisting of 6 extractors in series of the type described by Brown and Jones (*J.*, 1947, 1344). The first fraction, obtained by extraction with purified light petroleum (b. p. 40°), was purified by distillation in a vacuum giving fraction (8) (0·52 g.), b. p. 120°/1 mm., $n_D^{20°}$ 1·4524 (Found : OMe, 62·2. Calc. for tetramethyl methylgalactoside, $C_{11}H_{22}O_6$: OMe, 62·0%). The second fraction, obtained on continuing the extraction with ether, was added to the still residue and the distillation was resumed, yielding fraction (9) (1·50 g.), b. p. 140—160°/1 mm., $n_D^{23°}$ 1·4612 (Found : OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·3%). Concentration of the aqueous solution remaining in the extraction apparatus left a syrup which was added to the still residue and the distillation continued yielding fraction (10) (0·36 g.), b. p. 180°/1 mm., $n_D^{19°}$ 1·4710 (Found : OMe, 42·6. Calc. for $C_9H_{18}O_6$: OMe, 41·8%). The still residue (0·1 g.) was not further examined.

Fraction (8) (0·5 g.) was hydrolysed by boiling it with N-sulphuric acid (20 c.c.); $[\alpha]_D^{20°}$ + 110° (initial value), + 112° (final value, 4 hours). The sugar (0·41 g.), isolated in the usual manner, gave 2 : 3 : 4 : 6-tetramethyl D-galactose anilide, m. p. and mixed m. p. 189°, in almost quantitative yield. Fraction (9) (1·45 g.) was hydrolysed with boiling N-sulphuric acid (10 c.c.); $[\alpha]_D^{20°}$ − 8° (final value). The trimethyl sugar (1·2 g.) was isolated by continuous extraction of the neutralised solution with chloroform and had $[\alpha]_D^{20°}$ − 10° (*c*, 1·1 in water) (Found : OMe, 41·8%). This product was purified by distillation, and a syrup (1·1 g., b. p. 140°/0·1 mm., $n_D^{20°}$ 1·4720, OMe, 41·9%) was obtained which, on oxidation with bromine water, gave 2 : 3 : 6-trimethyl D-mannonolactone (0·91 g.), m. p. and mixed m. p. 81° (Found : OMe, 42·0%).

Fraction (10) (0·3 g.) was hydrolysed with boiling N-sulphuric acid for 4 hours; $[\alpha]_D$ + 26° ⟶ 70° (constant value). The sugar was isolated after evaporation of the neutralised solution to dryness under reduced pressure by extraction of the residue with boiling acetone (yield, 0·24 g.) (Found : OMe, 30%). It was then oxidised with bromine water giving a lactone which had $[\alpha]_D^{20°}$ + 64° ⟶ + 56° (3 days) (Found : equiv., 208. Calc. for dimethyl hexonic acid $C_8H_{14}O_6$: 206). The crude lactone was converted into the phenylhydrazide which had m. p. 170° (yield, 82%).

The samples of the crystalline phenylhydrazide of m. p. 170° (0·8 g.) from fractions (3), (7), and (10) were combined and hydrolysed by warming them with N-sodium hydroxide (5 c.c.) for 3 hours. Phenylhydrazine was then removed by solution in ether and the residual aqueous solution was acidified with N-sulphuric acid (5 c.c.). The 2 : 3-dimethyl D-mannonic acid (0·3 g.) was removed from the solution by continuous extraction with chloroform. On evaporation of the solvent, crystalline 2 : 3-dimethyl D-mannonolactone remained, m. p. 98°, raised to 107° on recrystallisation from acetone–ether–light petroleum; $[\alpha]_D^{20°}$ + 60° (*c* 1·1 in water, initial value), + 57° (24 hours), thereafter very slow change (Found : OMe, 30·1; equiv., 204. Calc. for $C_8H_{14}O_6$: OMe, 30·1%; equiv., 206). When the lactone was heated with alcoholic phenylhydrazine, the phenylhydrazide, m. p. 170°, was regenerated.

Oxidation of 2 : 3-*Dimethyl* D-*Mannonic Acid with Periodic Acid.*—The lactone (20 mg.) was warmed with N-sodium hydroxide (0·2 c.c.), and a solution of sodium periodate (excess) in N-sulphuric acid added. After 15 minutes at 40°, excess of solid potassium chloride was added, and the top clear aqueous layer decanted from the precipitated potassium iodate and periodate. The clear layer was mixed with an aqueous solution of phenylhydrazine hydrochloride and potassium ferricyanide, and on addition of a few drops of concentrated hydrochloric acid the port-wine colour diagnostic of the presence of formaldehyde was observed. A control experiment with 2 : 3 : 6-trimethyl D-mannonolactone gave no formaldehyde.

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DL-Phenylalanine
DL-Serine
DL-Tryptophane
L-Tyrosine
DL-Valine

Development work continues on L-glutamine, DL-lysine hydrochloride and DL-threonine, and also on β-alanine and sarcosine. It is expected that these and others will be ready for issue shortly.

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